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lovE Variant Regulator Molecules
(Atty Docket No. 109272.150; Client Docket No. MIC005US)

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BACKGROUND OF THE INVENTION

Field of the Invention

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The invention relates to the fields of microbiology and molecular biology. In particular, the invention relates to the field of mycology and the production of secondary metabolites from fungi.

Summary of the Related Art

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Secondary metabolites are a major source of commercially useful products such as food additives, vitamins, and medicines for the treatment of a wide variety of infections and diseases. By way of example, in 1997 the statin drugs lovastatin, simvastatin, and pravastatin, fungal secondary metabolites used in the treatment of hypercholesteremia, together had US sales of US\$7.53 billion (Sutherland et al., *Current Opinion In Drug Discovery & Development* 4:229-236 (2001)). The cost and availability of these plant, bacterial and fungal metabolites are frequently determined by limitations imposed on production and purification of these compounds from culture. This problem is frequently exacerbated by the fact that these products are generally produced during the stationary phase of bacterial and fungal growth.

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A wide variety of methods have been utilized to increase the amount of secondary metabolite produced in culture. Studies have demonstrated the importance of carefully designing the medium in which a fungus is grown to maximize the amount of a secondary metabolite produced (see, e.g., Hajjaj H, et al., *Appl. Environ. Microbiol.* 67:2596-602 (2001); Lesova, K., et al., *J. Basic Microbiol.* 40:369-75 (2000)). In addition, the method of culture or fermentation also impacts directly on the amount of secondary metabolite produced. For example, see Robinson, T., et al. (*Appl. Microbiol. Biotechnol.* 55:284-

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DATE OF DEPOSIT 10/9/01

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5 289 (2001)), which demonstrates the advantages of solid state (substrate) fermentation.

In addition to the manipulation of culture and media conditions, genetic approaches have been taken to increase secondary metabolite production. For example, the
10 production of penicillin is limited by the activity of two enzymes, encoded by the *ipnA* and *acvA* genes, both of which are regulated by the *pacC* protein, a zinc-finger transcription factor. Naturally occurring mutant alleles of the *pacC* locus are known to possess more transcription-
15 activating activity than the cognate, wild-type allele (see, e.g., Tilburn et al. *EMBO J.* 14(4):779-790 (1995)). Thus, one genetic approach to increasing secondary metabolite production is to identify and isolate naturally occurring mutant alleles, the expression of which leads to
20 increased secondary metabolite production.

Although many regulators of secondary metabolite production in many organisms are known, not all of the organisms that produce secondary metabolites are amenable to genetic or molecular genetic manipulation. Thus, these
25 systems are not generally useful as a source for the isolation of naturally occurring mutant alleles and are even less useful for the deliberate manipulation of secondary metabolite regulator protein structure with the aim of creating improved regulators of secondary
30 metabolite production.

It would be advantageous to have improved regulators of the biosynthetic enzymes responsible for secondary metabolite production. For example, recent studies suggest increasing usage of statin drugs, e.g., see Waters
35 D.D., *Am. J. Cardiol.* 88:10F-5F (2001)). Thus, demand for statin drugs is likely to increase substantially. In order to meet the demand for these and other secondary metabolites, new and improved methods for the production of secondary metabolites must be identified.

BRIEF SUMMARY OF THE INVENTION

The invention provides improved secondary metabolite regulator proteins that enable increased production of secondary metabolites. The invention also provides
10 methods to make these improved regulator proteins.

In a first aspect, the invention provides a variant regulator protein of secondary metabolite production with increased activity than that of the cognate, wild-type protein. In certain embodiments of this aspect of the
15 invention, the regulator protein is a fungal regulator protein.

In an embodiment of the first aspect, the invention provides an improved regulator protein comprising an amino acid sequence coding for a variant lovE protein having at
20 least one specific mutation that gives rise to greater transcription-activating properties of the regulator protein and/or induction of secondary metabolite synthesis.

By way of non-limiting example, certain preferred
25 regulator proteins of this aspect of the invention include at least one of the following mutations: (1) a Group 6 amino acid residue mutated to a Group 2 amino acid residue at position 31, in one embodiment the mutation represented by F31L; (2) a Group 3 amino acid residue mutated to a
30 Group 5 amino acid residue at position 41, in one embodiment the mutation represented by Q41K or Q41R; (3) a Group 4 amino acid residue mutated to a Group 2 amino acid residue at position 52, in one embodiment the mutation represented by T52I; (4) a Group 4 amino acid residue
35 mutated to a Group 3 amino acid residue at position 52, in one embodiment the mutation represented by T52N; (5) a Group 4 amino acid residue mutated to a Group 5 amino acid residue at position 73, in one embodiment the mutation represented by C73R; (6) a Group 1 amino acid residue
40 mutated to a Group 4 amino acid residue at position 101, in one embodiment the mutation represented by P101S; (7) a Group 1 amino acid residue mutated to a Group 3 amino acid residue at position 101, in one embodiment the mutation

5 represented by P101Q; (8) a valine amino acid residue
mutated to another Group 2 amino acid residue at position
111, in one embodiment the mutation represented by V111I;
(9) a Group 4 amino acid residue mutated to a Group 2
10 amino acid residue at position 133, in one embodiment the
mutation represented by S133L; (10) a Group 3 amino acid
residue mutated to a Group 2 amino acid residue at
position 141, in one embodiment the mutation represented
by E141V; (11) a Group 3 amino acid residue mutated to a
15 Group 5 amino acid residue at position 141, in one
embodiment the mutation represented by E141K; (12) a Group
4 amino acid residue mutated to Group 6 amino acid residue
at position 153, in one embodiment the mutation
represented by C153Y; (13) a Group 4 amino acid residue
20 mutated to a Group 5 amino acid residue at position 153,
in one embodiment the mutation represented by C153R; (14)
a Group 4 amino acid residue mutated to a Group 1 amino
acid residue at position 281, in one embodiment the
mutation represented by T281A; (15) a Group 3 amino acid
25 residue mutated to a Group 2 amino acid residue at
position 367, in one embodiment the mutation represented
by N367I; (16) a Group 3 amino acid residue mutated to a
Group 6 amino acid residue at position 367, in one
embodiment the mutation represented by N367Y; (17) a Group
30 1 amino acid residue mutated to Group 4 amino acid residue
at position 389, in one embodiment the mutation
represented by P389S; and (18) a Group 1 amino acid
residue mutated to a Group 2 amino acid residue at
position 389, in one embodiment the mutation represented
by P389L.

35 In some embodiments of the first aspect, the
invention provides regulator proteins with at least two,
or at least three, or at least four, or at least five, or
at least six, or at least seven, or at least eight, or at
least nine, or at least ten, or at least eleven, or at
40 least twelve, or at least thirteen, or at least fourteen,
or at least fifteen, or at least sixteen, or at least
seventeen, or at least eighteen of the above described
specific mutations.

5 In other embodiments of the first aspect, the invention provides an isolated lovE variant regulator protein selected from the group consisting of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID
 10 NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:65.

15 In a second aspect, the invention provides a nucleic acid molecule encoding a lovE regulator of the first aspect of the invention. By way of non-limiting example, the invention provides a nucleic acid molecule encoding the lovE variant regulator protein selected from the group
 20 consisting of SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:87,
 25 SEQ ID NO:88, SEQ ID NO:89, and SEQ ID NO:90.

In a third aspect, the invention provides a method of increasing the activity of a protein that regulates secondary metabolite production comprising: (a) selecting a nucleic acid comprising a polynucleotide encoding a
 30 protein regulator of secondary metabolite production; (b) mutating the nucleic acid to create a plurality of nucleic acid molecules encoding variant regulator proteins of secondary metabolite production; and (c) selecting a variant regulator protein with more activity than the
 35 cognate, wild-type protein.

In various embodiments of the third aspect, the secondary metabolite is a fungal secondary metabolite. In certain embodiments of the third aspect, the protein regulator of secondary metabolite production is a
 40 transcription factor. In certain embodiments of the third aspect, the protein regulator of secondary metabolite production is a transmembrane transporter, protein that

5 mediates secretion, kinase, G-protein, cell surface
receptor, GTPase activating protein, guanine nucleotide
exchange factor, phosphatase, protease, phosphodiesterase,
bacterial protein toxin, importin, RNA-binding protein,
SCF complex component, adherin, or protein encoded within
10 a biosynthetic cluster. In certain other embodiments of
the third aspect, the variant regulator protein is
selected to have more activity in a heterologous cell
and/or more activity in a homologous cell than the
cognate, wild-type regulator protein. In certain
15 embodiments, the variant regulator protein is selected to
have more activity in a heterologous cell and/or more
activity in a homologous cell than the cognate, wild-type
protein and to cause more secondary metabolite to be
produced in a homologous cell and/or a heterologous cell
20 when compared to the cognate, wild-type regulator protein.
In a particularly preferred embodiment, the variant
regulator protein is a lovE variant regulator protein.

In a fourth aspect, the invention provides a method
of increasing production of a secondary metabolite
25 comprising: (a) selecting a nucleic acid comprising a
polynucleotide encoding a protein regulator of secondary
metabolite production; (b) mutating the nucleic acid to
create a plurality of nucleic acid molecules encoding
variant regulator proteins of secondary metabolite
30 production; (c) selecting a variant regulator protein with
more activity than the cognate, wild-type protein; and (d)
expressing the selected variant regulator protein in a
cell, thereby increasing production of the secondary
metabolite in the cell.

35 In various embodiments of the fourth aspect, the
secondary metabolite is a fungal secondary metabolite. In
certain embodiments of the third aspect, the protein
regulator of secondary metabolite production is a
transcription factor. In certain embodiments of the
40 fourth aspect, the protein regulator of secondary
metabolite production is a transmembrane transporter, a

5 protein that mediates secretion, a kinase, a G-protein, a
cell surface receptor, a GTPase activating protein, a
guanine nucleotide exchange factor, a phosphatase, a
protease, a phosphodiesterase, a bacterial protein toxin,
an importin, an RNA-binding protein, an SCF complex
10 component, an adherin, or a protein encoded within a
biosynthetic cluster. In certain other embodiments of the
fourth aspect, the variant regulator protein is selected
to have more activity in a heterologous cell and/or more
activity in a homologous cell. In certain embodiments,
15 the variant regulator protein is selected to have more
activity in a heterologous cell and/or more activity in a
homologous cell and to cause more secondary metabolite to
be produced in a homologous cell and/or a heterologous
cell when compared to the cognate, wild-type regulator
20 protein. In a particularly preferred embodiment, the
variant regulator protein is a lovE variant regulator
protein.

In a fifth aspect, the invention provides an isolated
variant regulator protein of secondary metabolite
25 production having increased activity compared to a
cognate, wild-type protein, the variant regulator protein
made by the process comprising: (a) selecting a nucleic
acid comprising a polynucleotide encoding a protein
regulator of secondary metabolite production; (b) mutating
30 the nucleic acid to create a plurality of nucleic acid
molecules encoding variant regulator proteins of secondary
metabolite production; (c) selecting a variant regulator
protein with more activity than the cognate, wild-type
protein; and (d) recovering the selected variant regulator
35 protein.

In certain embodiments of the fifth aspect, the
secondary metabolite is a fungal secondary metabolite. In
certain embodiments of the fifth aspect, the protein
regulator of secondary metabolite production is a
40 transcription factor. In certain embodiments of the fifth
aspect, the protein regulator of secondary metabolite

5 production is a transmembrane transporter, a protein that
mediates secretion, a kinase, a G-protein, a cell surface
receptor, a GTPase activating protein, a guanine
nucleotide exchange factor, a phosphatase, a protease, a
phosphodiesterase, a bacterial protein toxin, an importin,
10 an RNA-binding protein, an SCF complex component, an
adherin, or a protein encoded within a biosynthetic
cluster. .In certain embodiments of the fifth aspect,
the variant regulator protein has more activity in a
heterologous and/or a homologous cell than the cognate,
15 wild-type protein. In certain embodiments of the fourth
aspect, the variant regulator protein increases production
of a secondary metabolite in a heterologous cell and/or a
homologous cell when compared to the cognate, wild-type
protein. In a particularly preferred embodiment, the
20 variant regulator protein is a lovE variant regulator
protein.

In a sixth aspect, the invention provides a fungus
having improved lovastatin production made by the process
of transforming a fungal cell with a nucleic acid molecule
25 encoding a lovE variant protein of the first aspect of the
invention. In an embodiment thereof, the nucleic acid
molecule is selected from a nucleic acid molecule of the
second aspect of the invention.

In a seventh aspect, the invention provides an
30 improved process for making lovastatin comprising
transforming a fungal cell with a nucleic acid molecule
encoding a variant of the lovE protein of the first aspect
of the invention. In an embodiment thereof, the fungal
cell is transformed with a nucleic acid molecule of the
35 second aspect of the invention.

In a eighth aspect, the invention provides a nucleic
acid molecule encoding a lovE protein defined by SEQ ID
NO:91. In an embodiment thereof, the invention provides
an isolated lovE nucleic acid molecule defined by SEQ ID
40 NO:92.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a photographic representation of cells growing on media with and without G418 selection demonstrating *lovFp-HIS3p-Neo* activation in *S. cerevisiae*. Controls include MB968 (vector only), MB2478 (lowly expressed wild-type *lovE*), and MB1644 (highly expressed wild-type *lovE*). All *lovE* variants are expressed in an MB968 vector backbone similar to MB2478.

Figure 2A is a graphic representation of *lovFp-CYC1p-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from the clones *lovE* 1-10.

Figure 2B is a graphic representation of *lovFp-CYC1p-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from the clones *lovE* 1-10 from a separate transformation than that of Figure 2A.

Figure 3 is a graphic presentation of *lovFp-CYC1p-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from clones *lovE* 16-41.

Figure 4 is a graphic presentation of *lovFp-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from clones *lovE* 1-10.

Figure 5 is a graphic presentation of *lovFp-lacZ* expression in *S. cerevisiae* strains expressing *lovE* variant proteins from clones *lovE* 16, 20, 21, 30-34, and 36-41.

Figure 6 is a graphic presentation of lovastatin culture concentration, as measured by enzyme inhibition assay, from broths of *A. terreus* cultures expressing *lovE* variant proteins 1-10 in.

Figure 7A is a graphic depiction of lovastatin culture concentration, as measured by HPLC analysis, from

5 broths of *A. terreus* cultures expressing lovE variant
proteins 1-10 in MF117.

Figure 7B is a graphic depiction of lovastatin
culture concentration, as measured by HPLC analysis, from
10 broths of *A. terreus* cultures expressing lovE variant
proteins 2, 6, 30, 32, 36, 37, 39, and 41 in MF117.

FIGURE 7B

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The patents and publications cited herein reflect the level of knowledge in the art and are hereby incorporated by reference in their entirety. Any conflict between any teaching of such references and this specification shall be resolved in favor of the latter.

The invention utilizes techniques and methods common to the fields of molecular biology, genetics and microbiology. Useful laboratory references for these types of methodologies are readily available to those skilled in the art. See, for example, Molecular Cloning, A Laboratory Manual, 3rd edition, edited by Sambrook, J., MacCallum, P., and Russell, D.W. (2001), Cold Spring Harbor Laboratory Press (ISBN: 0-879-69576-5); Current Protocols In Molecular Biology, edited by Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Struhl, K. (1993), John Wiley and Sons, Inc. (ISBN: 0-471-30661-4); PCR Applications: Protocols for Functional Genomics, edited by Innis, M.A., Gelfand, D.H., Sninsky, J.J. (1999), Cold Spring Harbor Press (ISBN: 0-123-72186-5); and Methods In Yeast Genetics, 2000 Edition: A Cold Spring Habor Laboratory Course Manual, by Burke, D., Dawson, D. and Stearns, T., Cold Spring Harbor Press (ISBN: 0-879-69588-9).

In certain embodiments of the aspects of the invention, the invention relates to the biosynthesis and improved production of secondary metabolites. The invention provides variant regulator proteins useful for the production of secondary metabolites, nucleic acid molecules encoding variant regulator proteins, and methods for their production.

In a first aspect, the invention provides a variant regulator protein of secondary metabolite production with increased activity relative to a cognate, wild-type regulator protein. Particularly preferred are variant regulator proteins of fungal secondary metabolites.

As used herein, the terms "fungal" and "fungus" refer generally to eukaryotic, heterotrophic organisms with an

5 absorptive mode of nutrition. Fungi typically contain
 chitin in their cell walls and exhibit mycelial or yeast-
 like growth habits (More Gene Manipulations in Fungi,
 edited by J.W. Bennet and L.L. Lasure, Academic Press Inc.
 (1991), ISBN 0120886421). More specifically, the terms
 10 refer to secondary metabolite producing organisms
 including, without limitation, *Aspergillus sp.*,
Penicillium sp., *Acremonium chrysogenum*, *Yarrowia*
lipolytica, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*,
Claviceps sp., *Trichoderma sp.*, *Tolypocladium sp.*,
 15 *Tricotheicium sp.*, *Fusidium sp.*, *Emericellopsis sp.*,
Cephalosporium sp., *Cochliobolus sp.*, *Helminthosporium*
sp., *Agaricus brunescens*, *Ustilago maydis*, *Neurospora sp.*,
Pestalotiopsis sp. and *Phaffia rhodozyma* (See, Fungal
Physiology, Chapter 9 (Secondary(Special) Metabolism),
 20 Griffin, D. H., John Wiley & Sons, Inc.; ISBN:
 0471166154).

The term "variant regulator protein" is used herein
 to refer to any regulatory protein having at least one
 change or difference in the amino acid sequence of the
 25 protein when compared to its cognate, wild-type regulatory
 protein sequence. The term does not include naturally
 occurring allelic variations of the cognate, wild-type
 regulatory protein.

The term "regulator protein" is meant to refer to a
 30 protein having a positive or negative function that
 modifies the production of a secondary metabolite. The
 function of the protein may be at the level of
 transcription, e.g., repression or activation, protein
 synthesis, or transport. The regulator may alter the
 35 level of transcription, RNA stability, translation, post-
 translational modification, or cellular localization of
 proteins involved in secondary metabolite synthesis and/or
 transport. The regulator may also have effects on
 precursor metabolite pools, flux through specific pathways
 40 and metabolite resistance.

By way of non-limiting example, certain embodiments
 of the aspects of the invention relate to a regulator
 protein that is a protein that contributes and/or promotes

5 transcription of a gene sequence, i.e., a transcription-activating protein. "Transcription-activating" is a term used to refer to characteristics of a protein that promote transcription. As used herein, a transcription-activating protein would include proteins that increase accessibility
 10 of the DNA to transcription complexes, for example, by opening or relaxing chromatin structure, proteins that promote the recognition and/or binding of transcription complexes to a target gene sequence, and/or proteins that promote transcription complex movement along the length of
 15 the template DNA sequence.

Regulatory proteins of secondary metabolite production and the nucleic acid sequences encoding these are known to those skilled in the art. Non-limiting examples of regulatory proteins of secondary metabolite
 20 synthesis include: regulator proteins of the aflatoxin/sterigmatocystin biosynthetic cluster (Woloshuk, C.P., et al., *Appl. Environ. Microbiol.* **60**:2408-2414 (1994) and Brown, D.W., et al., *Proc Natl Acad Sci U S A.* **93**:1418-1422 (1996)); regulator proteins of the paxilline
 25 biosynthetic cluster (Young, C., et al., *Mol. Microbiol.* **39**:754-764 (2001)); regulator proteins of the cephalosporin and penicillin biosynthetic clusters (Litzka O., et al., *Antonie Van Leeuwenhoek* **75**:95-105 (1999); Schmitt E.K. and Kuck U., *J. Biol. Chem.* **275**:9348-9357
 30 (2000); MacCabe et al. *Mol. Gen. Genet.* **250**:367-374 (1996); Suarez et al. *Mol. Microbiol.* **20**:529-540 (1996); Lambert et al. *Mol. Cell. Biol.* **17**:3966-3976 (1997); Su et al. *Genetics* **133**:67-77 (1993); regulator proteins of tricothecene synthesis (Trapp S.C., et al., *Mol. Gen.*
 35 *Genet.* **257**:421-432 (1998); Brown D.W., et al., *Fungal Genet. Biol.* **32**:121-133 (2001); and Matsumoto G., et al. *Biosci. Biotechnol. Biochem.* **63**:2001-2004 (1999)); and regulator proteins of lovastatin synthesis (Kennedy, J., et al., *Science* **284**:1368-1372 (1999); Hendrickson et al.,
 40 *Chem. Biol.* **6**:429-439 (1999) Tag, A. et al., *Mol Microbiol.* **38**:658-65 (2000)).

5 Certain embodiments of the aspects of the invention
disclosed herein relate to the lovE regulator protein, a
protein which plays a key role in the biosynthesis of
lovastatin. More particularly, certain embodiments of the
aspects of the invention relate to variant proteins of the
10 lovE regulator protein and methods of making the same.
Such proteins are variant with respect to the following A.
terreus wild-type lovE sequences (SEQ ID NOS:91 and 92).

Table 1: Amino Acid and Nucleic Acid Sequences of Wild-type lovE

Wild-type lovE Amino Acid Sequence

maadqgftnsvtlspvegsrtggtlprrafrsdcdrchaqkikctgnkevtgrapcqr
qqaglrvcysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvsseshsnts
rqfldppdsydwswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekap
lppvssdiaraasaqrelfddlsavsqeleellavtviewpkqeiwthpigmffnasrrl
ltvlrqqagadchqgtldeclrtknlfavhcyilnvriltaiselllsqirrtqnshms
plegsrsqsprrddtssssghssvdtipffsenlpigelfsyvdpplthalfsacttlhvg
vqllreneitlgvhsaggiaasismsgepgediartgatnsarceeqpttpaarvlfmfl
sdegafqeaksagsrgrtiaalrrcyedifslarkhkhgmlrdlnnipp (SEQ ID
NO:91)

Wild-type lovE DNA Sequence

atggctgcagatcaaggtatattcacgaactcggtcactctctcgccagtgagggttca
cgcaccggtggaacattaccccgccgtgcattccgacgctcttgatcggtgtcatgca
caaaagatcaaagtactggaaataaggaggttactggccgtgctccctgtcagcggtgc
cagcaggttgacttcgatgcgtctacagtgcgagcgatgccccaaagcgcaagctacgcaa
tccagggcagcggatctcgtctctgctgacccagatccctgcttgacatgtcctcgct
ccagtgcctcacagagcttgccgctagacgtatccgagtcgcatctcctcaaatacctcc
cggcaatttcttgatccaccggacagctacgactggctcgtggacctcgattggcactgac
gaggctattgacactgactgctgggggctgtcccaatgtgatggaggcttcagctgtcag
ttagagccaacgctgcccgatctaccttcgcccttcgagtcacggttgaaaaagctccg
ttgccaccggtatcgagcgacattgctcgtgcggccagtgcgcaacgagagcttttcgat
gacctgtcggcggtgtcgcaggaactggaagagatccttctggccgtgacggtagaatgg
ccgaagcaggaaatctggacccatcccatcggaatgtttttcaatgcgtcacgacggctt
cttactgtcctgcgccaacaagcgagccgactgccatcaaggcacactagacgaatgt
ttacggaccaagaacctctttacggcagctacactgttacatattgaatgtgcggattttg
accgccatatcgaggttgcctctgtcgaaattaggcggacccagaacagccatatgagc
ccactggaaggagtcgatccagtcgccgagcagagacgacaccagcagcagcagcggc
cacagcagtggttgacaccataaccttcttttagcgagaacctcctattgggtgagctgttc
tcctatgttgacccctgacacacgccctattctcggttgactacgttacatgttggg
gtacaattgctgcgtgagaatgagattactctgggagtagactccgcccagggcattgca
gcttccatcagcatgagcggggaaccaggcgaggatatagccaggacagggcgaccaat
tccgcaagatgcgaggagcagccgaccactccagcggctcgggttttgcattgttcttg
agtgatgaaggggctttccaggaggcaaagtctgctggttcccaggtcgaaccatcgca
gcactgcgacgatgctatgaggatatcttttccctcgcccgcaaacacaaacatggcatg
ctcagagacctcaacaatattcctccatga (SEQ ID NO:92)

15 As used herein, the term "secondary metabolite" means
a compound, derived from primary metabolites, that is
produced by an organism, is not a primary metabolite, is
not ethanol or a fusel alcohol, and is not required for
growth under standard conditions. Secondary metabolites

5 are derived from intermediates of many pathways of primary
metabolism. These pathways include, without limitation,
pathways for biosynthesis of amino acids, the shikimic
acid pathway for biosynthesis of aromatic amino acids, the
polyketide biosynthetic pathway from acetyl coenzyme A
10 (CoA), the mevalonic acid pathway from acetyl CoA, and
pathways for biosynthesis of polysaccharides and
peptidopolysaccharides. Collectively, secondary
metabolism involves all primary pathways of carbon
metabolism. Particularly preferred in embodiments of the
15 aspects of the invention are fungal secondary metabolites
(See, Fungal Physiology, Chapter 9 (Secondary(Special)
Metabolism), Griffin, D. H., John Wiley & Sons, Inc.;
ISBN: 0471166154).

20 "Secondary metabolite" also includes intermediate
compounds in the biosynthetic pathway for a secondary
metabolite that are dedicated to the pathway for synthesis
of the secondary metabolite. "Dedicated to the pathway
for synthesis of the secondary metabolite" means that once
the intermediate is synthesized by the cell, the cell will
25 not convert the intermediate to a primary metabolite.

"Intermediate compounds" also include secondary metabolite
intermediate compounds which can be converted to useful
compounds by subsequent chemical conversion or subsequent
biotransformation. As such, providing improved
30 availability of such intermediate compounds would still
lead to improved production of the ultimate useful
compound, which itself may be referred to herein as a
secondary metabolite. The yeast *Saccharomyces cerevisiae*
is not known to produce secondary metabolites.

35 The term "primary metabolite" means a natural product
that has an obvious role in the functioning of almost all
organisms. Primary metabolites include, without
limitation, compounds involved in the biosynthesis of
lipids, carbohydrates, proteins, and nucleic acids. The
40 term "increasing the yield of the secondary metabolite"
means increasing the quantity of the secondary metabolite
present in the total fermentation broth per unit volume of
fermentation broth or culture.

5 As used herein, the phrase "modulate production of a
secondary metabolite" refers to a positive or negative or
desirable change in one or more of the variables or values
that affect the process or results of production of the
primary or secondary metabolites in a liquid or solid
10 state fungal fermentation. These positive or negative or
desirable changes include, without limitation, an increase
or decrease in the amount of a primary or secondary
metabolite being produced (in absolute terms or in
quantity per unit volume of fermentation broth or per unit
15 mass of solid substrate); a decrease in the volume of the
broth or the mass/quantity of substrate required for the
production of sufficient quantities; a decrease in the
cost of raw materials and energy, the time of fermentor or
culture run, or the amount of waste that must be processed
20 after a fermentor run; an increase or decrease in the
specific production of the desired metabolite (both in
total amounts and as a fraction of all metabolites and
side products made by the fungus); an increase or decrease
in the percent of the produced secondary metabolite that
25 can be recovered from the fermentation broth or culture;
and an increase in the resistance of an organism producing
a primary or secondary metabolite to possible deleterious
effects of contact with the secondary metabolite.

In certain embodiments of aspects of the invention, a
30 secondary metabolite is an anti-bacterial. An "anti-
bacterial" is a molecule that has cytocidal or cytostatic
activity against some or all bacteria. Preferred anti-
bacterials include, without limitation, β -lactams.
Preferred β -lactams include, without limitation,
35 penicillins and cephalosporins and biosynthetic
intermediates thereof. Preferred penicillins and
biosynthetic intermediates include, without limitation,
isopenicillin N, 6-aminopenicillanic acid (6-APA),
penicillin G, penicillin N, and penicillin V. Preferred
40 cephalosporins and biosynthetic intermediates include,
without limitation, deacetoxycephalosporin V (DAOC V),
deacetoxycephalosporin C (DAOC), deacetylcephalosporin C

5 (DAC), 7-aminodeacetoxycephalosporanic acid (7-ADCA),
cephalosporin C, 7-B-(5-carboxy-5-oxopentanamido)-
cephalosporanic acid (keto-AD-7ACA), 7-B-(4-
carboxybutanamido)-cephalosporanic acid (GL-7ACA), and 7-
aminocephalosporanic acid (7ACA).

10 In certain embodiments of aspects of the invention,
the secondary metabolite is an anti-hypercholesterolemic
or a biosynthetic intermediate thereof. An "anti-
hypercholesterolemic" is a drug administered to a patient
diagnosed with elevated cholesterol levels for the purpose
15 of lowering the cholesterol levels. Preferred anti-
hypercholesterolemic include, without limitation,
lovastatin, mevastatin, simvastatin, and pravastatin.

According to other embodiments of the invention, a
secondary metabolite is an immunosuppressant or a
20 biosynthetic intermediate thereof. An "immunosuppressant"
is a molecule that reduces or eliminates an immune
response in a host when the host is challenged with an
immunogenic molecule, including immunogenic molecules
present on transplanted organs, tissues or cells.
25 Preferred immunosuppressants include, without limitation,
members of the cyclosporin family and beauverolide L.
Preferred cyclosporins include, without limitation,
cyclosporin A and cyclosporin C.

In certain embodiments of aspects of the invention,
30 the secondary metabolite is an ergot alkaloid or a
biosynthetic intermediate thereof. An "ergot alkaloid" is
a member of a large family of alkaloid compounds that are
most often produced in the sclerotia of fungi of the genus
Claviceps. An "alkaloid" is a small molecule that
35 contains nitrogen and has basic pH characteristics. The
classes of ergot alkaloids include clavine alkaloids,
lysergic acids, lysergic acid amides, and ergot peptide
alkaloids. Preferred ergot alkaloids include, without
limitation, ergotamine, ergosine, ergocristine,
40 ergocryptine, ergocornine, ergotaminine, ergosinine,
ergocristinine, ergocryptinine, ergocorninine, ergonovine,
ergometrinine, and ergoclavine.

5 In certain embodiments of aspects of the invention,
the secondary metabolite is an inhibitor of angiogenesis
or a biosynthetic intermediate thereof. An "angiogenesis
inhibitor" is a molecule that decreases or prevents the
formation of new blood vessels. Angiogenesis inhibitors
10 have proven effective in the treatment of several human
diseases including, without limitation, cancer, rheumatoid
arthritis, and diabetic retinopathy. Preferred inhibitors
of angiogenesis include, without limitation, fumagillin
and ovalicin.

15 In certain embodiments of aspects of the invention,
the secondary metabolite is a glucan synthase inhibitor or
a biosynthetic intermediate thereof. A "glucan synthase
inhibitor" is a molecule that decreases or inhibits the
production of 1,3- β -D-glucan, a structural polymer of
20 fungal cell walls. Glucan synthase inhibitors are a class
of antifungal agents. Preferred glucan synthase
inhibitors include, without limitation, echinocandin B,
pneumocandin B, aculeacin A, and papulacandin.

25 In certain embodiments of aspects of the invention,
the secondary metabolite is a member of the gliotoxin
family of compounds or a biosynthetic intermediate
thereof. The "gliotoxin family of compounds" are related
molecules of the epipolythiodioxopiperazine class.
Gliotoxins display diverse biological activities,
30 including, without limitation, antimicrobial, antifungal,
antiviral, and immunomodulating activities. Preferred
members of the "gliotoxin family of compounds" include,
without limitation, gliotoxin and aspirochlorine.

35 In certain embodiments of aspects of the invention,
the secondary metabolite is a fungal toxin or a
biosynthetic intermediate thereof. A "fungal toxin" is a
compound that causes a pathological condition in a host,
either plant or animal. Fungal toxins could be mycotoxins
present in food products, toxins produced by
40 phytopathogens, toxins from poisonous mushrooms, or toxins
produced by zoopathogens. Preferred fungal toxins
include, without limitation, aflatoxins, patulin,

5 zearalenone, cytochalasin, griseofulvin, ergochrome,
cercosporin, marticin, xanthocillin, coumarins,
tricothecenes, fusidanes, sesterpenes, amatoxins,
malformin A, phallotoxins, pentoxin, HC toxin, psilocybin,
10 bufotenine, lysergic acid, sporodesmin, pulcheriminic
acid, sordarins, fumonisins, ochratoxin A, and fusaric
acid.

With some certain embodiments of aspects of the
invention, the secondary metabolite is a modulator of cell
surface receptor signaling or a biosynthetic intermediate
15 thereof. The term "cell surface receptor" is as used
before. Modulators of cell surface receptor signaling
might function by one of several mechanisms including,
without limitation, acting as agonists or antagonists,
sequestering a molecule that interacts with a receptor
20 such as a ligand, or stabilizing the interaction of a
receptor and molecule with which it interacts. Preferred
modulators of cell surface signaling include, without
limitation, the insulin receptor agonist L-783,281 and the
cholecystokinin receptor antagonist asperlicin.

25 In certain embodiments of aspects of the invention,
the secondary metabolite is a plant growth regulator or a
biosynthetic intermediate thereof. A "plant growth
regulator" is a molecule that controls growth and
development of a plant by affecting processes that
30 include, without limitation, division, elongation, and
differentiation of cells. Preferred plant growth
regulators include, without limitation, cytokinin, auxin,
gibberellin, abscisic acid, and ethylene.

In certain embodiments of aspects of the invention,
35 the secondary metabolite is a pigment or a biosynthetic
intermediate thereof. A "pigment" is a substance that
imparts a characteristic color. Preferred pigments
include, without limitation, melanins and carotenoids.

In certain embodiments of aspects of the invention,
40 the secondary metabolite is an insecticide or a
biosynthetic intermediate thereof. An "insecticide" is a
molecule that is toxic to insects. Preferred insecticides
include, without limitation, nodulisporic acid.

5 In certain embodiments of aspects of the invention,
the secondary metabolite is an anti-neoplastic compound or
a biosynthetic intermediate thereof. An "anti-neoplastic"
compound is a molecule that prevents or reduces tumor
formation. Preferred anti-neoplastic compounds include,
10 without limitation, taxol (paclitaxel) and related
taxoids.

The phrase "increased activity" is used herein to
refer to a characteristic that results in an augmentation
of the inherent negative or positive function of the
15 regulatory protein.

The invention provides variant regulator proteins of
secondary metabolite production with increased activity
and methods of producing the same. The invention further
provides for the identification of specific amino acid
20 residues that are important to the functioning of
secondary metabolite regulator proteins. By way of non-
limiting example, variant regulator proteins of the
secondary metabolite regulator lovE are presented herein.

As known to those skilled in the art, certain
25 substitutions of one amino acid for another may be
tolerated at one or more amino acid residues of a wild-
type regulator protein absent a change in the structure,
activity and/or function of the wild-type protein. Such
substitutions are referred to in the art as "conservative"
30 substitutions, and amino acids may be categorized into
groups that identify which amino acids may be substituted
for another without altering the structure and/or function
of the protein.

As used herein, the term "conservative substitution"
35 refers to the exchange of one amino acid for another in
the same conservative substitution grouping in a protein
sequence. Conservative amino acid substitutions are known
in the art and are generally based on the relative
similarity of the amino acid side-chain substituents, for
40 example, their hydrophobicity, hydrophilicity, charge,
size, and the like. In a preferred embodiment,
conservative substitutions typically include substitutions
within the following groups: Group 1: glycine, alanine,

5 and proline; Group 2: valine, isoleucine, leucine, and methionine; Group 3: aspartic acid, glutamic acid, asparagine, glutamine; Group 4: serine, threonine, and cysteine; Group 5: lysine, arginine, and histidine; Group 6: phenylalanine, tyrosine, and tryptophan. Each group
 10 provides a listing of amino acids that may be substituted in a protein sequence for any one of the other amino acids in that particular group.

As stated *supra*, there are several criteria used to establish groupings of amino acids for conservative
 15 substitution. For example, the importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle, *Mol. Biol.* 157:105-132 (1982)). It is known that certain amino acids may be
 20 substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. Amino acid hydrophilicity is also used as a criteria for the establishment of conservative amino acid groupings (see, e.g., U.S. Patent No.
 25 4,554,101).

Information relating to the substitution of one amino acid for another is generally known in the art (see, e.g., Introduction to Protein Architecture : The Structural Biology of Proteins, Lesk, A.M., Oxford University Press;
 30 ISBN: 0198504748; Introduction to Protein Structure, Branden, C.-I., Tooze, J., Karolinska Institute, Stockholm, Sweden (January 15, 1999); and Protein Structure Prediction: Methods and Protocols (Methods in Molecular Biology), Webster, D.M. (Editor), August 2000,
 35 Humana Press, ISBN: 0896036375).

In one embodiment of the first aspect, the invention provides an improved regulator protein comprising an amino acid sequence coding for a variant of the lovE protein having at least one specific mutation that gives rise to
 40 greater transcription-activating properties of the regulator protein and/or increased lovastatin synthesis.

By way of non-limiting example, certain amino acid residues and mutations thereof in the lovE regulatory

5 protein of *A. terreus* (SEQ ID NO:91) are identified by the
invention described herein. Mutations at residues 31, 41,
52, 73, 101, 111, 133, 141, 153, 281, 367, and 389 of the
wild-type lovE protein of *A. terreus* have been identified
as being critical for the improvement of lovE regulator
10 protein function. Those mutations include: F31L, Q41K,
Q41R, T52I, T52N, C73R, P101S, P101Q, V111I, S133L, E141V,
E141K, C153Y, C153R, T281A, N367I, N367Y, P389S and P389L.
Each mutation, therefore, represents a change of one
conservative class of amino acids for another. For
15 example, the mutation F31L represents a change from a
Group 6 amino acid residue to a Group 2 amino acid residue
at position 31 of the wild-type, lovE regulator protein.

Thus, by way of non-limiting example, regulator
proteins of this aspect of the invention include at least
20 one of the following mutations: (1) a Group 6 amino acid
residue mutated to a Group 2 amino acid residue at
position 31, for example, the mutation represented by
F31L; (2) a Group 3 amino acid residue mutated to a Group 5
amino acid residue at position 41, for example, the
25 mutation represented by Q41K or Q41R; (3) a Group 4 amino
acid residue mutated to a Group 2 amino acid residue at
position 52, for example, the mutation represented by
T52I; (4) a Group 4 amino acid residue mutated to a Group
3 amino acid residue at position 52, for example, the
30 mutation represented by T52N; (5) a Group 4 amino acid
residue mutated to a Group 5 amino acid residue at
position 73, for example, the mutation represented by
C73R; (6) a Group 1 amino acid residue mutated to a Group
4 amino acid residue at position 101, for example, the
35 mutation represented by P101S; (7) a Group 1 amino acid
residue mutated to a Group 3 amino acid residue at
position 101, for example, the mutation represented by
P101Q; (8) a valine amino acid residue mutated to another
Group 2 amino acid residue at position 111, for example,
40 the mutation represented by V111I; (9) a Group 4 amino
acid residue mutated to a Group 2 amino acid residue at
position 133, for example, the mutation represented by
S133L; (10) a Group 3 amino acid residue mutated to a

- 5 Group 2 amino acid residue at position 141, for example, the mutation represented by E141V; (11) a Group 3 amino acid residue mutated to a Group 5 amino acid residue at position 141, for example, the mutation represented by E141K; (12) a Group 4 amino acid residue mutated to Group
- 10 6 amino acid residue at position 153, for example, the mutation represented by C153Y; (13) a Group 4 amino acid residue mutated to a Group 5 amino acid residue at position 153, for example, the mutation represented by C153R; (14) a Group 4 amino acid residue mutated to a
- 15 Group 1 amino acid residue at position 281, for example, the mutation represented by T281A; (15) a Group 3 amino acid residue mutated to a Group 2 amino acid residue at position 367, for example, the mutation represented by N367I; (16) a Group 3 amino acid residue mutated to a
- 20 Group 6 amino acid residue at position 367, for example, the mutation represented by N367Y; (17) a Group 1 amino acid residue mutated to Group 4 amino acid residue at position 389, for example, the mutation represented by P389S; and/or (18) a Group 1 amino acid residue mutated to
- 25 a Group 2 amino acid residue at position 389, for example, the mutation represented by P389L.

In other embodiments of the first aspect, the invention provides a variant of the lovE regulator protein with at least two, or at least three, or at least four, or

30 at least five, or at least six, or at least seven, or at least eight, or at least nine, or at least ten, or at least eleven, or at least twelve, or at least thirteen, or at least fourteen, or at least fifteen, or at least sixteen, or at least seventeen, or at least eighteen of

35 the above described specific mutations.

In other embodiments of the first aspect, the invention provides an isolated lovE variant regulator protein having the sequence of SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46,

40 SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58,

5 SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62,
SEQ ID NO:63, SEQ ID NO:64, or SEQ ID NO:65.

In a second aspect, the invention provides a nucleic acid molecule encoding a variant regulator protein of secondary metabolite production of the first aspect of the invention. As used herein, the terms "nucleic acid" or "nucleic acid molecule" refer to a deoxyribonucleotide or ribonucleotide polymer in either single-or double-stranded form, and unless otherwise limited, would encompass analogs of natural nucleotides that can function in a similar manner as the naturally occurring nucleotide.

In one embodiment of the second aspect, the invention provides a nucleic acid molecule encoding a variant protein of the lovE regulator protein of the first aspect of the invention.

20 By way of non-limiting example, the invention provides a nucleic acid molecule encoding a lovE variant regulator protein having the sequence of SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, or SEQ ID NO:90.

Poor transformation efficiency and the lack of efficient selection systems frequently precludes the screening of large numbers of variant regulator proteins of secondary metabolites in the organism from which the regulator protein is isolated. For example, there are currently certain technical obstacles to the successful screening of large numbers of variant regulator proteins in the fungus *A. terreus*, an organism that produces the secondary metabolite lovastatin.

The invention described herein takes advantage of the genetically tractable and experimentally amenable organism *Saccharomyces cerevisiae* for screening large numbers of variant regulator proteins of secondary metabolite production. Techniques common to the field of molecular biology are well developed in *S. cerevisiae*, and large

5 numbers of vectors are available to assist the genetic manipulation and cloning of variant regulator proteins involved in secondary metabolite production. Other genetically tractable organisms could also be used for this purpose.

10 In a third aspect, the invention provides a method of increasing the activity of a protein that regulates secondary metabolite production comprising: (a) selecting a nucleic acid comprising a polynucleotide encoding a protein regulator of secondary metabolite production; (b)
15 mutating the nucleic acid to create a plurality of nucleic acid molecules encoding variant regulator proteins of secondary metabolite production; and (c) selecting a variant regulator protein with more activity than the cognate, wild-type protein.

20 As used herein, "mutating" is used to refer to the deliberate alteration of at least one nucleotide residue of a wild-type, cognate nucleic acid sequence encoding a regulator protein of secondary metabolite production. A deliberate alteration or change in at least one nucleotide
25 residue of a polynucleotide may be accomplished by any method known in the art. The mutation(s) can be made *in vivo* or *in vitro* and can include random, partially random or not random, *i.e.*, directed, mutagenesis techniques.

By way of non-limiting example, *in vivo* mutagenesis
30 can be done by placing this nucleic acid molecule in a cell with a high mutation frequency, *i.e.* a mutagenic strain. By way of non-limiting example, Muhlrad *et al.* (Yeast 8:79-82 (1992)) have developed a rapid method for localized mutagenesis of yeast genes. As a first step,
35 the region of interest of a gene sequence is first amplified *in vitro* under error-prone polymerase chain reaction (PCR) conditions. Error-prone polymerase chain reaction (PCR) is a method of introducing amino acid changes into proteins. With this technique, mutations are
40 deliberately introduced during the PCR reaction through the use of error-prone DNA polymerases under specific reaction conditions. With the Muhlrad *et al.* procedure, the PCR product is then co-transformed with a gapped

5 plasmid containing homology to both ends of the PCR product, resulting in *in vivo* recombination to repair the gap with the mutagenized DNA.

There are a variety of commercially available kits that may be used to produce mutant nucleic acid molecules by error-prone PCR (see, e.g., GeneMorph™ PCR Mutagenesis Kit (Stratagene, La Jolla, California); and Diversify™ PCR Random Mutagenesis Kit (BD Biosciences Clontech, Palo Alto, CA). Thus, a plurality of variant, *i.e.*, mutated, regulator proteins of secondary metabolite production may be produced using established mutagenesis techniques.

As used herein, the term "activity" refers to a characteristic of the regulator protein that negatively or positively affects the biological system to bring about a modulation in secondary metabolite production. By way of non-limiting example, the activity is the transcription of downstream genes involved in the biosynthetic pathway of the secondary metabolite of choice. Thus, in the present example, the phrase "more activity" refers to the property of a variant regulator protein to bring about more transcription than that effected by the cognate, wild-type regulator protein.

In certain embodiments of the third aspect, the selected variant regulator protein has more activity in a fungal cell than the cognate, wild-type protein. In certain embodiments of the third aspect, the protein regulator of secondary metabolite production is a transcription factor. In certain embodiments of the fourth aspect, the protein regulator of secondary metabolite production is a transmembrane transporter, a protein that mediates secretion, a kinase, a G-protein, a cell surface receptor, a GTPase activating protein, a guanine nucleotide exchange factor, a phosphatase, a protease, a phosphodiesterase, a bacterial protein toxin, an importin, an RNA-binding protein, an SCF complex component, an adherin, or a protein encoded within a biosynthetic cluster. . In certain other embodiments of the third

5 aspect, the selected variant regulator protein has more activity in a heterologous cell than the cognate, wild-type protein. In certain embodiments thereof, the heterologous cell is an organism selected from the group consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*, *Candida*
 10 *sp.*, and *N. crassa*. In yet certain other embodiments of the third aspect, the selected variant regulator protein has more activity in a homologous cell than the cognate, wild-type protein. In certain embodiments thereof, the homologous cell is an organism selected from the group
 15 consisting of *Aspergillus sp.*, *Penicillium sp.*, *Acremonium chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*, *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*, *Tricotheicium sp.*, *Fusidium sp.*, *Emericellopsis sp.*, *Cephalosporium sp.*, *Cochliobolus sp.*,
 20 *Helminthosporium sp.*, *Agaricus brunescens*, *Ustilago maydis*, *Neurospora sp.*, *Pestalotiopsis sp.*, and *Phaffia rhodozyma*.

In certain embodiments of the third aspect, the selected variant regulator protein has more activity in a
 25 heterologous cell and a homologous cell than the cognate, wild-type protein. In certain embodiments thereof, the heterologous cell is an organism selected from the group consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*, *Candida sp.*, and *N. crassa* and the homologous cell is an organism
 30 selected from the group consisting of *Aspergillus sp.*, *Penicillium sp.*, *Acremonium chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*, *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*, *Tricotheicium sp.*, *Fusidium sp.*, *Emericellopsis sp.*,
 35 *Cephalosporium sp.*, *Cochliobolus sp.*, *Helminthosporium sp.*, *Agaricus brunescens*, *Ustilago maydis*, *Neurospora sp.*, *Pestalotiopsis sp.* and *Phaffia rhodozyma*.

As used herein, the phrase "heterologous cell" refers to a system for gene expression, i.e., an organism for
 40 gene expression, that is one other than the organism from which the selected regulator protein of secondary

5 metabolite production has been isolated. Preferred heterologous cells include, but are not limited to, *S. cerevisiae*, *E. coli*, *A. nidulans*, and *Candida sp.*, and *N. crassa*. Particularly preferred are fungal heterologous cells. In an embodiment of the third aspect, the method
 10 comprises: (a) selecting a nucleic acid comprising a polynucleotide encoding a protein regulator of secondary metabolite production; (b) mutating the nucleic acid to create a plurality of nucleic acid molecules encoding variant regulator proteins of secondary metabolite
 15 production; and (c) selecting a mutagenized nucleic acid encoding a variant regulator protein with increased activity in a homologous cell than the cognate, wild-type protein.

As used herein, the phrase "homologous cell" refers
 20 to a system for gene expression, i.e., an organism for gene expression, that is the organism from which the regulator protein of secondary metabolite production has been isolated. Preferred homologous cells are fungal homologous cells, including, but not limited to,
 25 *Aspergillus sp.*, *Penicillium sp.*, *Acremonium chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*, *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*, *Tricotheicium sp.*, *Fusidium sp.*, *Emericellopsis sp.*, *Cephalosporium sp.*, *Cochliobolus sp.*,
 30 *Helminthosporium sp.*, *Agaricus brunescens*, *Ustilago maydis*, *Neurospora sp.*, *Pestalotiopsis sp* and *Phaffia rhodozyma*. (See, Fungal Physiology, Chapter 9 (Secondary(Special) Metabolism), Griffin, D. H., John Wiley & Sons, Inc.; ISBN: 0471166154).

35 In certain embodiments of the third aspect, the method further comprises selecting a variant regulator protein that also increases production of a secondary metabolite in a cell when compared to the cognate, wild-type protein. In certain embodiments thereof, the cell is
 40 a fungal cell. In certain embodiments thereof, the cell is a heterologous cell, preferably selected from the group

5 consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*, *Candida*
sp., and *N. crassa*.

In certain embodiments thereof, the cell is a
homologous cell, preferably selected from the group
consisting of *Aspergillus sp.*, *Penicillium sp.*, *Acremonium*
10 *chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium sp.*,
Fusarium sp., *Monascus sp.*, *Claviceps sp.*, *Trichoderma*
sp., *Tolypocladium sp.*, *Tricotheicum sp.*, *Fusidium sp.*,
Emericellopsis sp., *Cephalosporium sp.*, *Cochliobolus sp.*,
Helminthosporium sp., *Agaricus brunescens*, *Ustilago*
15 *maydis*, *Neurospora sp.*, *Pestalotiopsis sp.*, and *Phaffia*
rhodozyma.

Certain embodiments of the aspects of the invention
relate to regulator proteins that promote secondary
metabolite production by increasing transcription of one
20 or more genes involved with secondary metabolite
production. These wild-type sequences may be selected for
mutagenesis to create a plurality of variant regulator
proteins. The activity of these transcription-activating
variant regulator proteins may be determined by measuring
25 the activity of a reporter gene having the appropriate
promoter sequences. These tests are done in a homologous
and/or a heterologous cell. Certain embodiments of
aspects of the invention are directed to fungal regulator
proteins with transcription-activating activity that is
30 tested in fungal heterologous and homologous cells.

Reporter genes are useful for isolating transformants
expressing improved variant regulator proteins. The
reporter genes may be operably linked to a promoter
sequence that is normally regulated by the wild-type
35 regulator protein. Reporter genes include, but are not
limited to, genes encoding β -galactosidase (*lacZ*), β -
glucoronidase (*GUS*), β -glucosidase, amylase and invertase,
amino acid biosynthetic genes, e.g., the yeast *LEU2*, *HIS3*,
LYS2, *TRP1* genes (or homologous genes from other fungi,
40 such as filamentous fungi, that encode proteins with the
similar functional activities), nucleic acid biosynthetic

5 genes, e.g., the yeast *URA3* and *ADE2* genes (or homologous
genes from other fungi, such as filamentous fungi, that
encode proteins with the similar functional activities),
the mammalian chloramphenicol transacetylase (CAT) gene,
or any surface antigen gene for which specific antibodies
10 are available. A reporter gene can also be a neomycin
phosphotransferase(neo) gene, which encodes neomycin,
kanamycin resistance gene and G418 (geneticin) resistance
gene. A reporter gene may encode a protein detectable by
luminescence or fluorescence, such as green fluorescent
15 protein (GFP). Reporter genes may additionally or
alternatively encode any protein that provides a
phenotypic marker, for example, a protein that is
necessary for cell growth or viability, or a toxic protein
that causes cell death. Alternatively, the reporter gene
20 may encode a protein detectable by a color assay leading
to the presence or absence of color.

The choice of reporter gene will depend on the type
of cell to be transformed. Preferred reporter genes are
those that are operable in fungal cells. It is preferable
25 to have two reporter genes within the cell. One reporter
gene, when expressed, provides a growth advantage to
transformed cells that are expressing the variant
regulator protein. This allows for the isolation of such
transformants though selective pressures. The other
30 reporter gene provides a colorimetric marker, such as the
lacZ gene and its encoded protein, β -galactosidase.
Alternatively, the second reporter provides a fluorescent
or luminescent marker, such as green fluorescent protein
(GFP).

35 In a fourth aspect, the invention provides a method
of increasing production of a secondary metabolite
comprising: (a) selecting a nucleic acid comprising a
polynucleotide encoding a protein regulator of secondary
metabolite production; (b) mutating the nucleic acid to
40 create a plurality of nucleic acid molecules encoding
variant regulator proteins of secondary metabolite
production; (c) selecting a variant regulator protein with

- 5 more activity than the cognate, wild-type protein; and (d) expressing the selected variant regulator protein in a cell, thereby increasing production of the secondary metabolite in the cell.

10 In certain embodiments of the fourth aspect, the cell is a fungal cell. In certain embodiments of the fourth aspect, the protein regulator of secondary metabolite production is a transcription factor. In certain
 15 embodiments of the fourth aspect, the protein regulator of secondary metabolite production is a transmembrane transporter, a protein that mediates secretion, a kinase, a G-protein, a cell surface receptor, a GTPase activating protein, a guanine nucleotide exchange factor, a
 20 phosphatase, a protease, a phosphodiesterase, a bacterial protein toxin, an importin, an RNA-binding protein, an SCF complex component, an adherin, or a protein encoded within a biosynthetic cluster. In certain embodiments of the fourth aspect, the cell is a heterologous cell, preferably selected from the group consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*, *Candida sp.*, and *N. crassa*. In certain
 25 other embodiments of the fourth aspect, the cell is a homologous cell, preferably selected from the group consisting of *Aspergillus sp.*, *Penicillium sp.*, *Acremonium chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*, *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*, *Tricotheicium sp.*, *Fusidium sp.*,
 30 *Emericellopsis sp.*, *Cephalosporium sp.*, *Cochliobolus sp.*, *Helminthosporium sp.*, *Agaricus brunescens*, *Ustilago maydis*, *Neurospora sp.*, *Pestalotiopsis sp.*, and *Phaffia rhodozyma*.

35 In certain other embodiments of the fourth aspect, the cell is a heterologous cell and the method further comprises expressing the variant regulator protein in a homologous cell, thereby increasing secondary metabolite production in the homologous cell. In certain embodiments
 40 thereof, the heterologous cell is an organism selected from the group consisting of *S. cerevisiae*, *E. coli*, *A.*

In certain embodiments of the fifth aspect, the variant regulator protein selected has more activity in a fungal cell. In certain embodiments of the fifth aspect, the protein regulator of secondary metabolite production is a transcription factor. In certain embodiments of the fifth aspect, the protein regulator of secondary metabolite production is a transmembrane transporter, a protein that mediates secretion, a kinase, a G-protein, a cell surface receptor, a GTPase activating protein, a guanine nucleotide exchange factor, a phosphatase, a protease, a phosphodiesterase, a bacterial protein toxin, an importin, an RNA-binding protein, an SCF complex component, an adherin, or a protein encoded within a biosynthetic cluster. In certain embodiments of the fifth aspect, the variant regulator protein selected has

5 more activity in a heterologous cell, preferably selected from the group consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*, *Candida sp.*, *Neurospora sp.*, *Pestalotiopsis sp.*, and *N. crassa*. In certain embodiments of the fifth aspect, the variant regulator protein selected has more
 10 activity in a homologous cell, preferably selected from the group consisting of *Aspergillus sp.*, *Penicillium sp.*, *Acremonium chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*, *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*, *Tricotheicium sp.*,
 15 *Fusidium sp.*, *Emericellopsis sp.*, *Cephalosporium sp.*, *Cochliobolus sp.*, *Helminthosporium sp.*, *Agaricus brunescens*, *Ustilago maydis*, *Neurospora sp.*, *Pestalotiopsis sp.*, and *Phaffia rhodozyma*.

In certain embodiments of the fifth aspect, the
 20 variant regulator protein selected has more activity in a homologous cell and a heterologous cell. In embodiments thereof, the heterologous cell is an organism selected from the group consisting of *S. cerevisiae*, *E. coli*, *A. nidulans*, *Candida sp.*, *Neurospora sp.*, *Pestalotiopsis sp.*,
 25 and *N. crassa* and the homologous cell is an organism selected from the group consisting of *Aspergillus sp.*, *Penicillium sp.*, *Acremonium chrysogenum*, *Yarrowia lipolytica*, *Nodulisporium sp.*, *Fusarium sp.*, *Monascus sp.*, *Claviceps sp.*, *Trichoderma sp.*, *Tolypocladium sp.*,
 30 *Tricotheicium sp.*, *Fusidium sp.*, *Emericellopsis sp.*, *Cephalosporium sp.*, *Cochliobolus sp.*, *Helminthosporium sp.*, *Agaricus brunescens*, *Ustilago maydis*, *Neurospora sp.*, *Pestalotiopsis sp.*, and *Phaffia rhodozyma*.

In yet another embodiment of the fifth aspect, the
 35 variant regulator protein is a variant protein of the lovE protein having at least one of the following mutations:
 (1) a Group 6 amino acid residue mutated to a Group 2 amino acid residue at position 31, for example, the mutation represented by F31L; (2) a Group 3 amino acid
 40 residue mutated to a Group 5 amino acid residue at position 41, for example, the mutation represented by Q41K

- 5 or Q41R; (3) a Group 4 amino acid residue mutated to a Group 2 amino acid residue at position 52, for example, the mutation represented by T52I; (4) a Group 4 amino acid residue mutated to a Group 3 amino acid residue at position 52, for example, the mutation represented by
- 10 T52N; (5) a Group 4 amino acid residue mutated to a Group 5 amino acid residue at position 73, for example, the mutation represented by C73R; (6) a Group 1 amino acid residue mutated to a Group 4 amino acid residue at position 101, for example, the mutation represented by
- 15 P101S; (7) a Group 1 amino acid residue mutated to a Group 3 amino acid residue at position 101, for example, the mutation represented by P101Q; (8) a valine amino acid residue mutated to another Group 2 amino acid residue at position 111, for example, the mutation represented by
- 20 V111I; (9) a Group 4 amino acid residue mutated to a Group 2 amino acid residue at position 133, for example, the mutation represented by S133L; (10) a Group 3 amino acid residue mutated to a Group 2 amino acid residue at position 141, for example, the mutation represented by
- 25 E141V; (11) a Group 3 amino acid residue mutated to a Group 5 amino acid residue at position 141, for example, the mutation represented by E141K; (12) a Group 4 amino acid residue mutated to Group 6 amino acid residue at position 153, for example, the mutation represented by
- 30 C153Y; (13) a Group 4 amino acid residue mutated to a Group 5 amino acid residue at position 153, for example, the mutation represented by C153R; (14) a Group 4 amino acid residue mutated to a Group 1 amino acid residue at position 281, for example, the mutation represented by
- 35 T281A; (15) a Group 3 amino acid residue mutated to a Group 2 amino acid residue at position 367, for example, the mutation represented by N367I; (16) a Group 3 amino acid residue mutated to a Group 6 amino acid residue at position 367, for example, the mutation represented by
- 40 N367Y; (17) a Group 1 amino acid residue mutated to Group 4 amino acid residue at position 389, for example, the mutation represented by P389S; and/or (18) a Group 1 amino acid residue mutated to a Group 2 amino acid residue at

5 position 389, for example, the mutation represented by P389L.

In certain embodiments of this aspect of the invention, the variant protein of the lovE protein sequence has an amino acid sequence of SEQ ID NO:41, SEQ
10 ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID
15 NO:62, SEQ ID NO:63, SEQ ID NO:64, or SEQ ID NO:65.

In another embodiment thereof, the variant protein of the lovE protein is encoded by a nucleic acid molecule having a polynucleotide sequence of SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID
20 NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, or SEQ ID NO:90.

25 In a sixth aspect, the invention provides a fungus having improved lovastatin production made by the process of transforming a fungal cell with a nucleic acid molecule encoding a variant of the lovE protein of the first aspect of the invention. In an embodiment thereof, the nucleic
30 acid molecule is selected from a nucleic acid molecule of the second aspect of the invention.

In a seventh aspect, the invention provides an improved process for making lovastatin comprising transforming a fungal cell with a nucleic acid molecule
35 encoding a variant of the lovE protein of the first aspect of the invention. In an embodiment thereof, the fungal cell is transformed with a nucleic acid molecule of the second aspect of the invention.

International Patent Application PCT/US99/29583
40 discloses lovastatin production genes. However, this reference does not provide a mature lovE cDNA sequence. The invention herein remedies the shortcoming of this

5 reference by providing a complete cDNA sequence for the *lovE* mRNA.

In an eighth aspect, the invention provides a nucleic acid molecule encoding a *lovE* protein defined by SEQ ID NO:91. In an embodiment thereof, the invention provides
10 an isolated *lovE* nucleic acid molecule defined by SEQ ID NO:92.

The following examples illustrate the preferred modes of making and practicing the present invention but are not meant to limit the scope of the invention since
15 alternative methods may be utilized to obtain similar results.

EXAMPLES

20 **Example 1: Preparation of Strains and Plasmids**

Strain MY2124 was derived from the Sigma 1278b strain background of *S. cerevisiae* and its complete genotype is as follows: *MAT α /MAT α ::LEU2 ura3 Δ 0 /ura3 Δ 0 leu2 Δ 0/leu2 Δ 0 trp1 Δ 0::*hisG*/trp1 Δ 0::*hisG* his3 Δ 0::*hisG*/his3 Δ 0::*hisG*
25 *ura3 Δ 0>::lovF-HIS3p-neo/ura3 Δ 0*. MY2124 can be constructed by mating *S. cerevisiae* strains MY2112 (*MAT α ura3 Δ 0 leu2 Δ 0 trp1 Δ 0::*hisG* his3 Δ 0::*hisG* ura3 Δ 0::*lovFp*-HIS3p-neo) with MY1555 (*mat α ::LEU2 ura3 Δ 0 leu2 Δ 0 trp1 Δ 0::*hisG* his3 Δ 0::*hisG*) and isolating zygotes. The *ura3 Δ 0>::lovFp-HIS3p-neo* allele of MY2112 was derived by cotransforming
30 *SfiI*-linearized plasmid MB2254 with pRS424 (Sikorski and Hieter (1989) *Genetics* 122:19-27) into MY1413 (*MAT α leu2 Δ 0 trp1 Δ 0::*hisG* his3 Δ 0::*hisG*). Transformants were selected on SC-Trp media and subsequently screened for 5-fluoro-orotic acid resistance to identify those
35 transformants containing the *ura3 Δ 0>::lovFp-HIS3p-neo* allele. Trp⁻ segregants lacking plasmid pRS424 were isolated by growing the strain under non-selective conditions.****

5 The following oligonucleotides were used in the construction of plasmids.

| Table 2: Oligonucleotides Utilized For <i>lovE</i> Variant Cloning | |
|---|---|
| MO664 | (5' GGCCATGGAGGCCGCTAGCTCGAGTCGACGGCCTAGGTGGCCAGCT3') (SEQ ID NO:1) |
| MO665 | (5' GGCCACCTAGGCCGTCGACTCGAGCTAGCGGCCTCCATGGCCGTAC3') (SEQ ID NO:2) |
| MO666 | (5' GCGGCCGCTCTAGAACTAGTCTCGAGGGTACC3') (SEQ ID NO:3) |
| MO667 | (5' GGTACCCCTCGAGACTAGTTCTAGAGCGGCCGCC3') (SEQ ID NO:4) |
| MO1794 | (5' CACAGCGGCCGCTCAACCTTCCCATTGGGGC3') (SEQ ID NO:5) |
| MO1793 | (5' CACCACTAGTACGCGGGCTGATTTCGAC3') (SEQ ID NO:6) |
| MO1785 | (5' CACCACTAGTTATACATTATATAAAGTAATGTG3') (SEQ ID NO:7) |
| MO1786 | (5' CACAGGATCCGTCATCTTTGCCTTCGTTTATC3') (SEQ ID NO:8) |
| MO195 | (5' CGCGGATCCTATTGAACAAGATGGATTGCAC3') (SEQ ID NO:9) |
| MO196 | (5' CCGGAATTCAGAAGAACTCGTCAAGAAG3') (SEQ ID NO:10) |
| MO841 | (5' ACAAAAAAGCAGGCTCCACAATGGCTGCAGATCAAGGTAT3') (SEQ ID NO:11) |
| MO842 | (5' ACAAGAAAGCTGGGTTCATGGAGGAATATTGTTGA3') (SEQ ID NO:12) |
| MO2278 | (5' GGGGATCCAATCGAGGTCCACGACCACT3') (SEQ ID NO:13) |
| MO343 | (5' GGGGACAAGTTTGTACAAAAAGCAGGCT3') (SEQ ID NO:14) |
| MO2273 | (5' GGGGATCCGCCAATGGTCCCGTTCAAAC3') (SEQ ID NO:15) |
| MO2274 | (5' ACAAGAAAGCTGGGTTCACAGAATGTTTAGCTCAA3') (SEQ ID NO:16) |
| MO344 | (5' GGGGACCACTTTGTACAAGAAAGCTGGGT3') (SEQ ID NO:17) |
| MO2624 | (5' GCGATGCCCAAGCGCAAGCTACGCCAATCCAGGG3') (SEQ ID NO:18) |
| MO2654 | (5' CGTCGCGCCATTCGCCATTCAGGCTGCGCAACTGT3') (SEQ ID NO:19) |
| MO2680 | (5' GGACCTTTGCAGCATAAATTACTATACTTCT3') (SEQ ID NO:20) |
| MO2686 | (5' GCGCGTCCATTCGCCATTCAGGCTGCGCAACTGT3') (SEQ ID NO:21) |
| MO2681 | (5' TAAACTCTTGTTTTCTTCTTTCTCTAAAT3') (SEQ ID NO:22) |
| MO2700 | (5' CAGTGAGCGCGCGTAATACGACTCACTATAGGGCGA3') (SEQ ID NO:23) |
| MO2701 | (5' ATACTTCTATAGACACACAAACACAAATACACACAC3') (SEQ ID NO:24) |
| MO107 | (5' CGCGGATCCCGTCGTTTTACAAC3') (SEQ ID NO:25) |
| MO197 | (5' CCAAGCTTATTATTTTGTACACCAGACCAA3') (SEQ ID NO:26) |
| MO1293 | (5' GGAAGATCTAGCATCGTGGCCAATTTCTTCTAGTTT3') (SEQ ID NO:27) |
| MO1294 | (5' ATAAGAATGCGGCCGCTCAACCTTCCCATTGGGGCGTTTGC3') (SEQ ID NO:28) |
| MO1787 | (5' CACAGGATCCAGCATTATTAATTTAGTGTGTGTATTT3') (SEQ ID NO:29) |
| MO1788 | (5' CACCACTAGTCTCGAGCAGATCCGCCAG3') (SEQ ID NO:30) |
| MO1793 | (5' CACCACTAGTACGCGGGCTGATTTCGAC3') (SEQ ID NO:31) |
| MO1794 | (5' CACAGCGGCCGCTCAACCTTCCCATTGGGGC3') (SEQ ID NO:32) |
| MO511 | (5' GGCCATCGATACAAGTTTGTACAAAAAGCTGAAC3') (SEQ ID NO:33) |
| MO540 | (5' GCGGCCCTATTACACCACTTTGTACAAGAAAGC3') (SEQ ID NO:34) |
| MO1985 | (5' CACACGTCTCCGGCCTCAACCTTCCCATTGGGGCG3') (SEQ ID NO:35) |

| |
|---|
| NO:35) |
| MO1986 (5' CACACAGATCTCGTGGCCAATTTCTTCTAGTTTGA3') (SEQ ID NO:36) |
| MO1992 (5' CACACGGATCCACAATGTTACGTCCTGTAGAAACCCC3') (SEQ ID NO:37) |
| MO1993 (5' CACAGCGGCCGCTTCATTGTTTGCCTCCCTGCTG3') (SEQ ID NO:38) |
| MO316 (5' GCGGCCGCGGCGCCCGGCCCATGTCAACAAGAAT3') (SEQ ID NO:39) |
| MO318 (5' CCGCGGCCGAGTGGAGATGTGGAGT3') (SEQ ID NO:40) |

5

Plasmid MB2254 contains the *lovFp-HIS3p-neo* reporter gene flanked by *URA3* sequence. First primers MO664 (SEQ ID NO:1) and MO665 (SEQ ID NO:2) were annealed and inserted into the *KpnI-SacI* sites of plasmid pBluescript II KS (Stratagene,). The resulting vector, MB1038, contains a *SalI* site in the polylinker. Next, the *SpeI-XhoI* fragment from pJL164 (Brachmann et al. *Yeast* 14:115-132 (1998)) containing a deletion of the *URA3* gene with additional flanking sequences was inserted into the *NheI-SalI* sites of MB1038 to create MB1053. Primers MO666 (SEQ ID NO:3) and MO667 (SEQ ID NO:4) that contain multiple restriction sites (*NotI*, *XbaI*, *SpeI*, *XhoI* and *KpnI*) were then annealed together and ligated into the *SmaI* site of MB1053 to create MB1054. Next, the following four fragments were combined in MB1054 to obtain plasmid MB2254. The *lovF* promoter from *A. terreus* genomic DNA was PCR amplified with MO1794 (SEQ ID NO:5) and MO1793 (SEQ ID NO:6) and inserted into MB1054 on a *NotI-SpeI* fragment.

The *HIS3* basal promoter from pRS403 (Sikorski and Hieter, *Genetics* 122:19-27 (1989)) was PCR amplified with primers MO1785 (SEQ ID NO:7) and MO1786 (SEQ ID NO:8) and inserted into MB1054 on a *SpeI-BamHI* fragment. Finally, the *neo* gene (PCR amplified with MO195 (*BamHI*) (SEQ ID NO:9) and MO196 (*EcoRI*) (SEQ ID NO:10) from plasmid pYX11 (Xiao and Weaver, *Nucl. Acids Res.* 25:2985-2991 (1997)) and *CYC1* terminator sequences (*XhoI-KpnI* fragment from pRS426-*GAL-S* (Mumberg, et al., *Nucl. Acids. Res.* 22:5767-5768 (1994)) were first combined in pRS416 (Sikorski and Hieter,

5 Genetics 122:19-27 (1989)) and then cut out with *Bam*HI-
KpnI and inserted into MB1054 to create MB2254.

The *lovFp-HIS3p-neo* reporter in MY2124 can confer resistance to the drug geneticin (G418). It was empirically determined that MY2124 (untransformed or transformed with parental plasmids MB2478 (*CYC1-lovE/CEN*) or MB2848 (*CYC1-lovE/At274/CEN*) was unable to grow on YPD media supplemented with 100 µg /ml G418. Plasmid MB2478 contains the *CYC1* promoter operationally linked to the entire *A. terreus lovE* open reading frame. The *CYC1* promoter is a relatively weak promoter and thus the *lovE* ORF in MB2478 was expressed at low levels. MB2478 was the parental vector plasmid for creating full length *lovE* variants. Plasmid MB2848 contains the *CYC1* promoter operationally linked to a chimeric open reading frame consisting of the *A. terreus lovE* DNA binding domain fused to the carboxy-terminal portion of the *At274* gene (U.S. Serial No. 60/257,431, filed December 22, 2000).

MB2848 was used to create *lovE* variants in which the DNA binding domain was not mutated. Both MB2478 and MB2848 contain yeast CEN and autonomously replicating sequences and both are maintained at 1-2 copies per cell. In contrast to strains transformed with MB2478 or MB2848, strains transformed with plasmid MB1644 (*TEF1-lovE*/2 micron) were able to grow on G418-supplemented YPD media. The *lovE* gene of MB1644 is under control of the constitutively strong *S. cerevisiae TEF1* promoter. MB1644 contains a 2-micron origin for high-copy replication in yeast. An objective of these studies was to identify *lovE* variants which when expressed at low levels could confer G418 resistance similar to the highly expressed wild-type *lovE* molecule of MB1644. *S. cerevisiae* expression vectors used in these studies were constructed as follows.

MB968 is a low copy *S. cerevisiae* URA3 based expression vector. MB968 was created by inserting the
40 *EcoRV* fragment (containing the destination cassette) from gateway pEZC7201 (Invitrogen™, Carlsbad, CA) into

5 XhoI/SalI (filled in with Klenow) linearized pRS416 CYC1 (Mumberg, et al., Gene 156:119-122 (1995)).

MB1644 and MB2478 are URA3-based *S. cerevisiae* expression plasmids that contain the wild-type *lovE* gene. They are both derivatives of MB1199. MB1199 was created
 10 by using primers MO841 (SEQ ID NO:11) and MO842 (SEQ ID NO:12) to amplify the *lovE* ORF from *A. terreus* cDNA. Gateway (Invitrogen™, Carlsbad, CA) Cloning Technology (US Patent 5,888,732) was used to clone the *lovE* PCR fragment into the gateway entry vector pDONR206 (Invitrogen™,
 15 Carlsbad, CA) to create MB1199. Similarly, Gateway Cloning Technology was used to transfer the *lovE* ORF from MB1199 into MB968 to create MB2478 and into MB969 (U.S. Serial No. 60/198,335, filed April 18, 2000) to create MB1644.

20 MB2848 is a derivative of MB968 that contains a *lovE*-AT274 chimera. The *lovE* portion of MB2848 was derived by using oligos MO841 (SEQ ID NO:11) and MO2278 (SEQ ID NO:13) to PCR amplify the *lovE* DNA binding domain from *A. terreus* cDNA. A second round of PCR was performed with
 25 primers MO343 (SEQ ID NO:14) and MO2278 to add appropriate Gateway Cloning Technology compatible sequences. The At274 portion of MB2848 can be derived by using primers MO2273 (SEQ ID NO:15) and MO2274 (SEQ ID NO:16) to PCR amplify the carboxy-terminal domain of At274 from *A.*
 30 *terreus* cDNA. A second round of PCR was performed with primers MO344 (SEQ ID NO:17) and MO2273 to add appropriate Gateway Cloning Technology compatible sequences. The *lovE* and At274 PCR products were cut with *Bam*HI and purified over a QIAquick PCR purification kit (Qiagen, Valencia,
 35 CA) according to manufacturer's instructions. Finally, the products were mixed 3-4 hours in a standard ligation reaction and used in Gateway entry and destination reactions to create MB2848.

Gateway cloning technology was used to clone the *lovE*
 40 variants of interest into plasmid MB1419 which is a filamentous fungal expression vector. The MB1419 fungal selection marker is the *A. nidulans* *GPD* promoter controlling the *ble* gene from *S. hindustanus*. The

5 transgene is controlled by the *A. nidulans* PGK promoter.
A. terreus strain MF117 is a derivative of A. terreus
strain ATCC 20542.

Example 2: PCR Mutagenesis of the *lovE* DNA Binding Domain

10 The zinc finger DNA binding domain of *lovE* is encoded by
nucleotides 100-201 (SEQ ID NO:92). Oligos MO2624 (SEQ ID
NO:18) and MO2654 (SEQ ID NO:19) were used to PCR amplify
a *lovE* containing fragment from plasmid MB2478. The 1.7
kb product contains nucleotides 212-1410 of *lovE* and ~500
15 bp of flanking vector sequence. Two rounds of standard
PCR (1.5 mM MgCl₂) were performed with Amplitaq DNA
polymerase (Applied Biosystems, Foster City, Ca) according
to the manufacturer's instructions.

Plasmid MB2848 was cut with *KpnI*-*BamHI* to release a 1.1
20 kb fragment containing the *At274* portion of the *lovE*-*At274*
chimeric open reading frame. The remaining 5.5 kb vector
sequence retains the *lovE* DNA binding domain.

Example 3: PCR Mutagenesis of the *lovE* Open Reading Frame

25 *lovE* open reading frame insert was prepared according
to the following procedure. Oligo pairs MO2680 (SEQ ID
NO:20) /MO2686 (SEQ ID NO:21), MO2681 (SEQ ID NO:22)
/MO2686, and MO2700 (SEQ ID NO:23) /MO2701 (SEQ ID NO:24)
were used to PCR amplify the entire *lovE* open reading
30 frame from plasmid MB2478. The PCR products differ in the
amount of 5' and 3' vector sequence flanking the *lovE* open
reading frame.

PCR was performed using a GeneMorph PCR mutagenesis
kit (Stratagene, La Jolla, Ca) according to manufacturer's
35 instructions to achieve medium and high range mutation
frequencies.

Plasmid MB2478 was cut with *Asp718*/*XbaI* to release a
1.7 kb fragment. The remaining 5.0 kb vector sequence
completely lacks *lovE* ORF sequence.

40

Example 4: Transformation and Selection for G418R Isolates

5 All PCR products were purified using a QIAquick PCR purification kit (Qiagen) according to manufacturer's instructions. All vectors were gel purified using a QIAquick gel extraction kit (Qiagen) according to manufacturer's instructions.

10 The mutagenesis strategy of Muhlrad et al. (Yeast 8:79-82 (1992)) was used which involves cotransforming a mutated PCR product and gapped plasmids into *S. cerevisiae*, and then screening for *in vivo* recombinants having the desired phenotype).

15 Transformation of *Saccharomyces cerevisiae* was accomplished by the lithium acetate/single-stranded carrier DNA/polyethylene glycol (LiAc/ss-DNA/PEG) protocol (Woods R.A. and Gietz R.D. *Methods Mol. Biol.* 177:85-97 (2001)) with a 1:5 molar ratio of vector:insert DNA to generate >55,000 *in vivo* recombinant transformants on SC-Ura plates. Transformants were transferred by replica printing to YPD plates containing 100 µg/ml G418 and allowed to grow for 2-4 days at 30°C (Figure 1).

20 Drug resistant clones were confirmed in secondary assays including growth on G418 concentrations up to 2000 µg/ml. The plasmid-dependence of the phenotype was determined by observing the re-appearance of drug sensitivity correlating with loss of the library plasmid. *lovE* variant plasmids were recovered from promising candidates (Hoffman and Winston (1986) *Gene* 57:267). More than 70 *lovE* variants were identified and definitively characterized by DNA sequence and/or restriction digestion analysis.

30 Table 3 summarizes the G418 resistance phenotype and sequence analysis of 26 of these variants.

Table 3: Variant lovE Mutations

| <i>lovE</i> allele | <i>lovFp- neo</i> Mediated G418R | MO oligos used for random PCR mutagenesis | Amino Acid Change | 1 | Amino Acid Change | 2 | Amino Acid Change | 3 | Amino Acid Change | 4 | Amino Acid Change | 5 | Amino Acid Change | 6 | Amino Acid Change | 7 | Amino Acid Change | 8 | Amino Acid Change | 9 | Amino Acid Change | 10 | Amino Acid Change | 11 |
|-----------------------|--|--|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|----|-------------------------|----|
| 1 | -/+ | 2624/2654 | H253R | | S341P | | | | | | | | | | | | | | | | | | | |
| 2 | +/- | 2624/2654 | R121W | | S133L | | S322G | | | | | | | | | | | | | | | | | |
| 3 | +++ | 2624/2654 | C73R | | A83V | | T135I | | | | | | | | | | | | | | | | | |
| 4 | ++ | 2624/2654 | C73R | | E177G | | | | | | | | | | | | | | | | | | | |
| 5 | ++ | 2624/2654 | C73R | | | | | | | | | | | | | | | | | | | | | |
| 6 | +/- | 2624/2654 | C153Y | | E197K | | T281A | | | | | | | | | | | | | | | | | |
| 7 | + | 2624/2654 | C73R | | T256A | | N466S | | | | | | | | | | | | | | | | | |
| 8 | +++ | 2624/2654 | C73R | | E141V | | | | | | | | | | | | | | | | | | | |
| 9 | ++ | 2624/2654 | C73R | | E303K | | | | | | | | | | | | | | | | | | | |
| 10 | +++ | 2624/2654 | Q41K | | | | | | | | | | | | | | | | | | | | | |
| 16 | +++ | 2680/2686 | Q41K | | P16A | | G23S | | T9M | | Q362E | | | | | | | | | | | | | |
| 19 | +/- | 2700/2701 | R21H | | S34A | | Q80H | | A84S | | E303D | | H374D | | A440T | | A441V | | | | C445S | | P469S | |
| 20 | + | 2700/2701 | F31L | | T409I | | | | | | | | | | | | | | | | | | | |
| 21 | +++ | 2700/2701 | F31L | | M97I | | E113D | | D146N | | P163S | | N367I | | H458Y | | | | | | | | | |
| 30 | +/- | 2681/2686 | I43V | | Q295L | | | | | | | | | | | | | | | | | | | |
| 31 | ++ | 2680/2686 | F31L | | P101S | | C153R | | C159S | | E162K | | R293L | | S311N | | | | | | | | | |
| 32 | ++ | 2680/2686 | L14I | | E18V | | G138C | | E338G | | V361L | | P389S | | N400S | | | | | | | | | |
| 33 | ++ | 2680/2686 | Q41R | | S174Y | | A402T | | | | | | | | | | | | | | | | | |
| 34 | ++ | 2680/2686 | F31L | | T52I | | P101Q | | P108S | | V111I | | | | | | | | | | | | | |
| 36 | +/- | 2700/2701 | D85N | | I143F | | M232I | | T315I | | S382Y | | M385K | | | | | | | | | | | |
| 37 | ++ | 2700/2701 | T46I | | Q62R | | K77R | | S323C | | N367Y | | V373I | | | | | | | | | | | |
| 38 | +/- | 2700/2701 | Q41R | | T294I | | P310L | | G337D | | P389L | | A394V | | G436S | | | | | | | | | |
| 39 | + | 2680/2686 | T52N | | V111I | | T139 | | V184I | | T281A | | | | | | | | | | | | | |
| 40 | +++ | 2680/2686 | Q41R | | D4E | | V87I | | D110E | | E141K | | A189T | | N276D | | T347R | | N367I | | Q377R | | A425T | |
| 41 | +/- | 2680/2686 | D131N | | S133L | | R312G | | A429G | | | | | | | | | | | | | | | |
| wild- type | - | N/A | N/A | | | | | | | | | | | | | | | | | | | | | |

5

Table 4 summarizes amino acid substitutions that were isolated multiple times, suggesting that they are particularly important for improving *lovE* variant activity on *lovFp-HIS3p-neo* expression.

10

Table 4: *lovE* Mutations Isolated Multiple Times

| Amino Acid Change | Number of Times Isolated in <i>lovE</i> 1-41 | <i>lovE</i> variant |
|--------------------------|---|----------------------------|
| F31L | 4 | 20, 21, 31, 34 |
| Q41K | 2* | 10, 16 |
| Q41R | 3* | 33, 38, 40 |
| T52I/T52N | 1 each | 34, 39 |
| C73R | 6* | 3, 4, 5, 7, 8, 9 |
| P101S/P101Q | 1 each | 31, 34 |
| V111I | 2 | 34, 39 |
| S133L | 2 | 2, 41 |
| E141V, E141K | 1 each | 8, 40 |
| C153Y/C153R | 1 each | 6, 31 |
| T281A | 2 | 6, 39 |
| N367I/N367Y | 2/1 | 21, 40, 37 |
| P389S/P389L | 1 each | 32, 38 |

* allele was isolated in additional *lovE* variants that were not fully sequenced

Example 5: Increased *lovF-lacZ* Expression in *S. cerevisiae*

In order to quantify the increase in *lovF* expression, β -galactosidase activity was measured in *lovE* variant transformed *S. cerevisiae* strains that also harbored *lovFp-lacZ* reporter derivative plasmids. *lovF-lacZ* reporter derivative plasmids were constructed as follows.

Plasmid MB1918 contains the *lovFp-lacZ* reporter gene. It can be derived from pRS424 (Sikorski and Hieter (1989) *Genetics* 122:19-27). First, primers MO107 (SEQ ID NO:25) and MO197 (SEQ ID NO:26) are used to PCR amplify the *lacZ* gene from Yep355 (Myers, et al., *Gene* 45:299-310 (1986)). This *lacZ*-containing fragment was inserted into the *Bam*HI-*Hind*III sites of pRS416 (Sikorski and Hieter, *Genetics* 122:19-27 (1989)). This same *lacZ* fragment can be cut out of the resulting vector with *Kpn*I-*Not*I and inserted into the same sites of pRS424 to create pRS424-*lacZ*. Primers MO1293 (SEQ ID NO:27) and MO1294 (SEQ ID NO:28) are used

5 to PCR amplify a 2.09 kb fragment of the *lovF* promoter from *A. terreus* genomic DNA. The *lovF* promoter fragment was then cut with *NotI*-*BglII* and inserted into *NotI*-*BamHI* linearized pRS424-*lacZ*.

10 Plasmid MB2114 contains the *lovFp*-CYC1p-*lacZ* reporter gene. It can be derived from pRS424-*lacZ* (see MB1918 plasmid construction). Primers MO1787 (SEQ ID NO:29) and MO1788 (SEQ ID NO:30) are used to amplify the 264 bp basal CYC1 element from pRS415 CYC1 (Mumberg, et al., *Gene* 156:119-122 (1995)). This 264 bp fragment was inserted 15 upstream of the pRS424-*lacZ* derivative which has been digested with *SpeI*-*BamHI*. Finally, the *lovF* promoter from MB1918 was PCR amplified with MO1793 (SEQ ID NO:31) and MO1794 (SEQ ID NO:32) and inserted into the *NotI*-*SpeI* sites to create MB2114.

20 Yeast strains utilized in this study include strains MY2145 and MY2159, which are both derived from the *S. cerevisiae* sigma 1278b strain background; the genotypes are both strains are as follows: *MATa ura3Δ0 leu2Δ0 his3Δ::hisG trp1Δ0::hisG*. MY2145 and MY2159 contain the 25 *lovFp*-*lacZ* reporter plasmids MB2114 and MB1918, respectively.

MY2124 transformed with individual *lovE* variant plasmids was mated to *S. cerevisiae* strains MY2154 and MY2159. Diploids were selected on SC-UraTrp media. 30 Multiple diploids from each individual mating were assayed for *lovFp*-*lacZ* expression using 96 well format β -galactosidase assays. For β -galactosidase assays, cells were transferred from transformation plates to 96-well microtiter plates containing 200 μ l Z buffer. 12 strains 35 were transferred simultaneously using a 12-channel multi-pipettor to scoop cells from transformation plates. Duplicate samples were prepared for all assays. OD₆₀₀ readings were taken on samples in Z buffer. These values were used to normalize for equal cell number in all 40 assays. After determining OD₆₀₀, 150 μ l of each sample in

5 Z buffer was transferred onto a Millipore Multiscreen Assay System (Nitrocellulose Immobilon NC), filtered, and then washed by filtering 200 μ l Z buffer. 100 μ l Z buffer with β ME and detergents was then added to each well, as was 20 μ l 4 mg/ml ONPG. Reactions were incubated at 30°C, 10 stopped with 50 μ l 1 M Na₂CO₃, filtered into a polystyrene 96-well assay plate, and OD₄₂₀ was determined for each assay well. β -galactosidase units were determined using the Miller formula (O.D. 420 X 1000)/ (OD600*minutes*volume in mL). Z buffer is made by 15 dissolving the following in 1 L of water (16.1 g Na₂HPO₄-7H₂O, 5.5g NaH₂PO₄-H₂O, 0.75 g KCl and 0.246 g MgSO₄-7H₂O). Z buffer with detergents and β ME is made as follows: 9.8 ml Z buffer, 100 μ l 20 mg/ml CTAB, 100 μ l 10 mg/ml sodium deoxycholate, and 69 μ l β ME Control plasmids utilized in 20 these studies included MB968, MB2478 and MB1644.

Results of these studies are presented in Figures 2-5, demonstrating increased transcription-activating properties of the *lovE* variants disclosed herein.

25 **Example 6: Secondary Metabolite Production**

Transformation of filamentous fungi was performed according to the following procedure. Protoplasts were generated by inoculating rich media with spores. Spores were allowed to germinate for about 20 hrs or until germ 30 tubes were between 5 and 10 spore lengths. The germlings were centrifuged and washed twice with sterile distilled water and once with 1 M magnesium sulfate. Germlings were then resuspended in 1M magnesium sulfate containing approximately 2 mg/ml of Novozyme. Tubes were then 35 incubated at 30°C shaking at 80 RPM for about 2 hrs or until most of the hyphae were digested and protoplasts were abundant. Protoplasts were filtered through one layer of Miracloth. At least one volume of STC was added and protoplasts were centrifuged. Protoplasts were washed

5 twice with STC. Protoplasts then were resuspended in 1ml
STC and counted in a hemacytometer. A final concentration
of approximately 5×10^7 protoplasts/ml were frozen in a
9:1:0.1 solution of STC, SPTC and DMSO in a Nalgene Cryo
cooler at -80°C (cools $-1^\circ\text{C}/\text{min}$).

10 Solutions for transformation were as follows: STC
(0.8 M Sorbitol, 25 mM Tris-HCl pH 7.5, 25 mM CaCl_2) and
SPTC (0.8 M Sorbitol, 40% PEG 4000, 25 mM Tris-HCl pH 8,
50 mM CaCl_2). Transformation was accomplished according to
the following protocol. 1-5 μg of DNA comprising a *lovE*
15 variant according to the invention in a fungal expression
vector was placed in a 50 ml Falcon tube. 100 μl of
previously frozen protoplasts were added to the DNA,
gently mixed, and then incubated on ice for 30 min. 15 μl
of SPTC was added, followed by mixing by tapping and
20 incubation at RT for 15 min. 500 μl SPTC was added and
mixed well by tapping and rolling, then incubated at RT
for 15 min. 25 mls of regeneration minimal medium was
added, mixed well and poured on plates containing 25 mls
of regeneration minimal medium with 2X the concentration
25 of selection drug.

Transformation plates were incubated at 26°C for 5-6
days or until colonies started to appear. Regeneration
minimal medium contains trace elements, salts, 25 mM
sodium nitrate, 0.8 M Sucrose, and 1% agarose at pH 6.5.
30 The selection drug that was used successfully with *A.*
terreus is phleomycin, a broad-spectrum glycopeptide
antibiotic. Transformants were picked onto new plates
with a toothpick (if the fungus was sporulating) or with
sterile forceps (if the fungus did not sporulate).
35 Purification plates contained minimal medium (same as
regeneration minimal medium but containing 2 % instead of
0.8 M sucrose) and 1X drug concentration. Picked
transformants were incubated at 26°C for 5-6 days.

Transformants were grown in production media for
40 secondary metabolite production. Briefly, for *A. terreus*
and lovastatin production, spores were used as the
inoculum. Spores were obtained from the purification

5 plate by using a wooden inoculation stick. The medium was RPM containing corn steep liquor, sodium nitrate, potassium phosphate, magnesium sulfate, sodium chloride, P2000 (Dow chemical), trace elements and lactose or glucose as carbon source. The medium was pH 6.5. Flasks
 10 were incubated at 26°C with shaking at 225 RPM. For static 96-well cultures, the same medium was used and the spores were obtained from the purification plate with a wooden toothpick. 96-well plates were incubated, without shaking at 26°C.

15 Sampling was done after after 5 days for lovastatin. For shake flask experiments 1-1.5 mls of supernatant was placed into 96-well plates, which were centrifuged and supernatants transferred to new 96-well plates. Samples were frozen at -80°C for storage or for
 20 later assays.

Cultures that were grown standing in a 96-well plate were centrifuged and the supernatant was transferred to a new 96 well plate. Samples were frozen at -80°C.

25 **Example 7: Measurement of Secondary Metabolite Production**

The concentration of the secondary metabolite lovastatin was determined by enzyme inhibition assay (Figure 6). Briefly, 10 µL of sample was removed and diluted 1:100 in H₂O. 10 µl of this diluted broth was
 30 assayed in a reaction (200 µL total) containing 1 mM HMGCoA, 1 mM NADPH, 0.005 mM DTT and 5 µl (His)₆HMGR. The disappearance of absorbance at 340 nm was observed over time. This represents the disappearance of NADPH, and lovastatin inhibits this reaction.

35 The initial velocities were calculated for the reactions containing samples, adjusted for dilution, and compared to reactions containing lovastatin standards to determine levels of metabolite produced. (His)₆HMGR was expressed in *Saccharomyces cerevisiae* and purified with a
 40 nickel column.

The results from ten individual transformants for each allele are shown in standard box plot format in

5 Figure 6. Lovastatin concentration from the corresponding
wild-type *lovE* control is shown in matching fill pattern.
For example, *lovE* alleles 2, 7, 8 and 9 were all
transformed and assayed at the same time as the non-
hatched wild-type control. The horizontal line in each
10 individual box represents the median.

Lovastatin concentration was also determined by high
pressure liquid chromatography (HPLC). Briefly, 100 μ L of
broth sample was removed and diluted 1:10 into 70% H₂O-30%
acetonitrile (900 μ l). This mixture was spun down to
15 pellet debris at 13000 RPM for 5 minutes. 900 μ l of this
diluted broth was transferred to a vial and the sample was
analyzed by HPLC. 10 μ l were injected into a Waters HPLC
system (996 photo-diode array detector, 600 E pump
controller and 717 autosampler) equipped with a YMC-Pack
20 ODS column (Aq-302-3, 150 x 4.6 mm ID, S-3 μ M pore size)
and eluted with isocratic 40% aqueous acetic acid (0.7%)-
60% acetonitrile for 8 minutes. Lovastatin was detected
at 238 nm to have a retention time of 6.5 minutes and was
quantified using a calibration curve created from pure
25 lovastatin samples.

The results from ten individual transformants for
each *lovE* variant are shown in standard box plot format in
Figure 7A and 7B. Thirty individual wild-type *lovE*
transformants and ten individual MB2143 negative control
30 transformants were tested. Identical controls are plotted
in Figures 7A and 7B.

PCR analysis of *A. terreus* transformants demonstrates
that greater than fifty percent of the transformants
contain the transgene. Variability in levels of transgene
35 expression can presumably be influenced by integration
site and copy number. *lovE* variants containing identical
amino acid substitutions are labeled.

The amino acid and nucleic acid sequences of *lovE*
variant sequences are presented in Table 5 and Table 6,
40 respectively.

Table 5: Amino Acid Sequences of Variants of the *lovE* Gene***lovE-1***

maadqgiftnsvtlspvegsrtggglprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfpyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrldlnnipp (SEQ ID NO:41)

lovE-2

maadqgiftnsvtlspvegsrtggglprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dwlwtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrldlnnipp (SEQ ID NO:42)

lovE-3

maadqgiftnsvtlspvegsrtggglprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserpkrklrqsrvadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswwisigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrldlnnipp (SEQ ID NO:43)

lovE-4

maadqgiftnsvtlspvegsrtggglprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserpkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvgkaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrldlnnipp (SEQ ID NO:44)

lovE-5

maadqgiftnsvtlspvegsrtggglprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserpkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrldlnnipp (SEQ ID NO:45)

lovE-6

maadqgiftnsvtlspvegsrtggglprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaidtdcwglsgydgffscqleptlpdlpspfestvekaplppvssdiaraasaq
rklddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvrilaaaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrldlnnipp (SEQ ID NO:46)

love-7

maadqgiftnsvtlspvegsrtggltprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserprkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaiddcwglsqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgaldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnsipp (SEQ ID NO:47)

love-8

maadqgiftnsvtlspvegsrtggltprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserprkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaiddcwglsqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgaldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnsipp (SEQ ID NO:48)

love-9

maadqgiftnsvtlspvegsrtggltprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserprkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaiddcwglsqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgaldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnsipp (SEQ ID NO:49)

love-10

maadqgiftnsvtlspvegsrtggltprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvyserprkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaiddcwglsqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgaldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnsipp (SEQ ID NO:50)

love-16

maadqgifmnsvtlsavegsrtsgtlprrafrrrscdrchakkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaiddcwglsqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmspldgsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:51)

love-19

maadqgiftnsvtlspvegshtggltprrafraccdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrhrsrasdlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaiddcwglsqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmspldgsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvdsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtitvlrrsyedifslark
hkhgmlrdlnnips (SEQ ID NO:52)

love-20

maadqgiftnsvtlspvegsrtggltprralrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvshssntsrqfldppdsy
dswtsigtdeaiddcwglsqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdpplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpittpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:53)

love-21

maadqgiftnsvtlspvegsrtggtlprralrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvsshsntsrqfldppdsy
dswtsigtdeaidtncwglsgcdggfscqlestlpldspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklnftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceepppttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:54)

love-30

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkvkctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvsshsntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpldspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklnftavhcyilnvriltaiselllsqirrtlnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceepppttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:55)

love-31

maadqgiftnsvtlspvegsrtggtlprralrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmsspsvpsqslpldvsshsntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfssqkptlpldspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklnftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqspnrrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceepppttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:56)

love-32

maadqgiftnsvtlspvvgstggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvsshsntsrqfldppdsy
dswtsictdeaidtdcwglsgcdggfscqleptlpldspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklnftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigglfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgesg
ediartgatssarceepppttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:57)

love-33

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrcharkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslpldvsshsntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpldspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklnftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnstrceepppttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:58)

love-34

maadqgiftnsvtlspvegsrtggtlprralrrscdrchaqkikctgnkevigrapcqrccqagl
rcvysercpkrklrqsraadlvsadpdpclhmssppvpsqslsldiseshsntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpldspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtnklnftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceepppttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:59)

love-36

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrccqagl
rcvysercpkrklrqsraanlvsadpdpclhmssppvpsqslpldvsshsntsrqfldppdsy
dswtsigtdeafdtcwglsgcdggfscqleptlpldspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigiffnasrrlltvlrqqagadchqgtldec
lrtnklnftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddisssssghssvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaayisksgepg
ediartgatnsarceepppttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmlrdlnnipp (SEQ ID NO:60)

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrchaqkikcignkevtgrapcqrqragl
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dswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsgeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchggtldec
lrtnknlftavhcyilnvriltaiselllsqirrtqnshmsplegsrsqsprrddtssssghscvd
tipffsenlpigelfsyvdplthalfsacttlhvgvqllreyeitlgihsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmldrlnnipp (SEQ ID NO:61)

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrcharkikctgnkevtgrapcqrccqagl
rcvysercpkrklrgsraadlvvadpdpclhmssppvpqspldvsseshsntsrqfldppdsy
dswtsigtdeaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleieillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchggtldec
lrtknflftavhcyilnvriltaiselllsqirriqnshmsplegsrsqslsrdtssssghssvd
tipffsenlpidelfsyvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgelg
edivrtgatnsarceeqpttpaarvlfmflsdegafqeaksagsrsrtiaalrrcyedifslark
hkhgmldrldnnipp (SEQ ID NO:62)

maadqgiftnsvtlspvegsrtggtlprrafrsrscdrchaqkikctgnkevngrapcqrccqag1
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dswtsigideaidthdcwglsgcdggfscqleptlpdlpspfestvekaplppissdiaraasag
relfddlsavsqeleieillavtvewpkqeiwthpigmffnasrrlltvlrqqagadchqgtldec
lrtknlfavhcyilnvrilaaaiselllsqirrtqnshmsplegrrsqsprrddtssssghssvd
tipffsenlpigefsvdplthalfsacttlhvgvqllreneitlgvhsaggiaasismsgepg
ediartgatnsarceeqpttpaarvlfsmflsdegafqeaksagsrgrtiaalrrcyedifslark
hkhgmrlrdlnnipp (SEQ ID NO:63)

maaeqgftnsvtlspvegsrtggtlprrafrrrscdrcharkikctgnkevtgrapcqrccqag1
rcvysercpkrklrgsraadlisadpdpclhmssppvpsqslplevseshsntsrqfldppdsy
dswtsigtDKaidtdcwglsgcdggfscqleptlpdlpspfestvekaplppvssditraasaq
relfddlsavsqeleieillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchqgtldec
lrtnknlftavhcyildvriltaisellsqirrtqnshmsplegsrsqsprrddtssssghssvd
tipffsenlpigefsvydprrhalsfacttlhvgvqllreieitlgvhsargiaasismsgepg
ediartgatnsarceeqpttpaarvflsdegtfqeaksagsrgrtiaalrrcyedifslark
hkhgmrlrdlnnipp (SEQ ID NO:64)

maadqgiftnsvtlspvegsrtggtlprrafrrrscdrchaqkikctgnkevtgrapcqrcqqag1
rcvysercpkrklrgsraadlvsadpdpclhmssppvpqslpldvsseshssntsrqfldppdsy
nwlwtsigtdeaiddtcwglsqcdggfscqleptlpdlpspfestvekaplppvssdiaraasaq
relfddlsavsqeleeillavtvewpkqeiwthpigmffnasrrlltvlrqqaqadchggtldec
lrtlknltavhcyilnvriltaisellsqirrtgnshmsplegrrsqspsgddtssssghssvd
tipffsenlpgelfsyvdlplthalfsacttlhvgvqllreneitlgvhsaqqiaasismsgpg
ediartgatnsarcceqpttpaarvlfmslfsdegafqegksagsrgrtiaalrrcyedifslark
hkhgmrlrdlnnipp (SEQ ID NO:65)

Table 6: DNA Sequences of Variants of the *lovE* Gene**lovE-1**

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGGCAGTTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCGTCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTA CTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:66)

lovE-2

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCTGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGTGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACGGCAGTGTGAC
 ACCATACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTA CTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:67)

lovE-3

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGTAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGATCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTA CTCTGGGAGTACACTCCGCCCAGGGCATTGCGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:68)

lovE-4

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
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 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTA CTCTGGGAGTACACTCCGCCCAGGGCATTGCGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:69)

love-5

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
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 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCAGGGCATTCAGCTTCCATCAGCATGAGCGGGGAACAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCGCAAGATGCGAGGAGCAGCCGACTACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:70)

love-6

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATATGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
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 CGAAAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
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 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
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 GAGGATATAGCCAGGACAGGGGCGACCAATTCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:71)

love-7

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 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
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 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCTCTCGCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
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 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
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 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCGCACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAGTATTCTCCATGA (SEQ ID NO:72)

love-8

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCTCTCGCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGTGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTGTCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:73)

love-9

ATGGCTGCAGATCAAGGTATATTCACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTCACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGACGCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 TTCTGCTGACCCAGATCCCTGCTTGCACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCAC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTGTCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGCAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAACGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGAAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:74)

love-10

ATGGCTGCAGATCAAGGTATATTCACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTCACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCAAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTGTCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGCAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGCTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:75)

love-16

ATGGCTGCAGATCAAGGTATATTCATGAACTCGGTCACTCTCTCTGCAGTGGAGGGTTACGCAC
 CAGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCAAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACAGTTGAAAAAGCTCCGTGTCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTATCGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACTAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTAGAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:76)

love-19

ATGGCTGCAGATCAAGGTATATTCACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACACAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCGCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCATTCCAGGGCATCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTGTCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGACG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCCTCCCTATTGGTGAGCTATTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTAGACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCACAGTACTGCGACGAAGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTTCATGA (SEQ ID NO:77)

love-20

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCACTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGCAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCACTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCAACCAATTCGCAAGATGCGAGGAGCAGCCGATCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAATATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:78)

love-21

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCACTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATATCCTCGCCTCCAGTGCCCTCACAGAGCTTACCGC
 TAGACGTATCCGATTTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTAACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGTCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGCAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCTAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGATTGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCACTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCAACCAATTCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAATATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:79)

love-30

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTATGCACAAAAGGTCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTGTCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCTGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:80)

love-31

ATGGCTGCAGATCAAGGTATATTACGAACTCCGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTACGACGCTCTTGTGATCGGTGTATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGTTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCTCTCGCCTTCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ACGTGATGGAGGCTTCAGCTCTCAGTTAAAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTGTCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTGCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTACTGTGCGAAATTAGGCTGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAACAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:81)

lovE-32

ATGGCTGCAGATCAAGGTATATTCACTAACTCGGTCACTATCTCGCCAGTGGTGGGTTCACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCTCGCTCCAGTGCCCTCACAGAGTTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTGGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGGGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGCTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAATCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAGTTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:82)

5

lovE-33

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACGAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCTCGCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCTCAATACCTCCCGGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 ATACGGTTGAAAAAGCTCCGTGGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGGGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCACAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:83)

lovE-34

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTGCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTATTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTATACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCAAGTGCCCTCACAGAGCTTGTCGC
 TAGACATATCCGAGTCGCATTCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGGCATTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:84)

lovE-36

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCACCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGAATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTACACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTTTTGACACTGACTGCTGGGGGCTATCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGCTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATCTTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAGCAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACATCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTTGCAGCTTACATCAGCAAGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTGTGTTTCATGTTCTTGAGTGATGAAGGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:85)

love-37

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTATTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAACGGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAGGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGCAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCTTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCTCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCGGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCTGTGTCGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGTATGAGA
 TTAATCTGGAATACTCCGCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTGCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGATCTCAACAATATTCCTCCATGA (SEQ ID NO:86)

love-38

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACGAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAAGCTGGACTT
 CGATGCGTCTATAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGCTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGCAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGATCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCTGAGCAGAGACGACACCAGCAGCAGTAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGATGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTAATCTGGGAGTACACTCCGCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCTAGGC
 GAGGATATAGTCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAAGTCAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:87)

love-39

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCACCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTAATGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCCCAGAGCTTGCCGC
 TAGACATATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCATTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAGT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGATATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGGCCGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAC
 ACACGCCCTATTCTCGGCTTGCACTACGTTACATGTTGGGGTACAATTGCTGCGTGAGAATGAGA
 TTACTCTGGGAGTACACTCCGCCCAGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGCTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:88)

love-40

ATGGCTGCAGAAACAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACGAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGTGTCTACAGTGAGCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCAT
 CTCTGCTGACCCAGATCCCTGCTTGACATGTCTCGCCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGAAGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 GACTGGTTCGTGGACCTCGATTGGCACTGACAAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTTGAGT
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 CGAGAGCTTTTCGATGACCTGTCGGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAGAATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGGATGTGCGGATTTTGAACGC
 CATATCGGAGTTGCTCCTGTGCGAAATTAGGCGGACCCAGAACAGCCATATGAGCCCACTGGAAG
 GGAGTCGATCCCAGTCGCCGAGCAGAGACGACACCAGCAGCAGCAGCGGCCACAGCAGTGTGAC
 ACCATACCCTTCTTTAGCGAGAACCTCCCTATTGGTGAGCTGTTCTCCTATGTTGACCCCTGAG
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 TTACTCTGGGAGTACACTCCGCCCGGGGCATTGCAGCTTCCATCAGCATGAGCGGGGAACCAGGC
 GAGGATATAGCCAGGACAGGGGCGACCAATTCCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTCATGTTCTTGAGTGATGAAGGGACTTTCCAGGAGGCAAAGTCTGCTGGTT
 CCCGAGGTCGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCTCCATGA (SEQ ID NO:89)

lovE-41

ATGGCTGCAGATCAAGGTATATTACGAACTCGGTCACTCTCTCGCCAGTGGAGGGTTCACGCAC
 CGGTGGAACATTACCCCGCCGTGCATTCCGACGCTCTTGTGATCGGTGTCATGCACAAAAGATCA
 AATGTACTGGAAATAAGGAGGTTACTGGCCGTGCTCCCTGTCAGCGTTGCCAGCAGGCTGGACTT
 CGATGCGTCTACAGTGAGCGATGCCCAAGCGCAAGCTACGCCAATCCAGGGCAGCGGATCTCGT
 CTCTGCTGACCCAGATCCCTGCTTGCACATGTCTCTACCTCCAGTGCCCTCACAGAGCTTGCCGC
 TAGACGTATCCGAGTCGCATTCCCTCAAATACCTCCCGCAATTTCTTGATCCACCGGACAGCTAC
 AACTGGTTGTGGACCTCGATTGGCACTGACGAGGCTATTGACACTGACTGCTGGGGGCTGTCCCA
 ATGTGATGGAGGCTTCAGCTGTCAGTTAGAGCCAACGCTGCCGGATCTACCTTCGCCCTTCGAAT
 CTACGGTTGAAAAAGCTCCGTTGCCACCGGTATCGAGCGACATTGCTCGTGCGGCCAGTGCGCAA
 CGAGAGCTTTTCGATGACCTGTGCGCGGTGTCGAGGAACTGGAAGAGATCCTTCTGGCCGTGAC
 GGTAAGATGGCCGAAGCAGGAAATCTGGACCCATCCCATCGGAATGTTTTCAATGCGTCACGAC
 GGCTTCTTACTGTCCTGCGCCAACAAGCGCAGGCCGACTGCCATCAAGGCACACTAGACGAATGT
 TTACGGACCAAGAACCTCTTTACGGCAGTACACTGTTACATATTGAATGTGCGGATTTTGACCGC
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 GAGGATATAGCCAGGACAGGGGCGACCAATTCGCAAGATGCGAGGAGCAGCCGACCACTCCAGC
 GGCTCGGGTTTTGTTTATGTTCTTGAGTGATGAAGGGGCTTCCAGGAGGGAAAGTCTGCTGGTT
 CCCGAGGTGGAACCATCGCAGCACTGCGACGATGCTATGAGGATATCTTTCCCTCGCCCGCAA
 CACAAACATGGCATGCTCAGAGACCTCAACAATATTCCTCCATGA (SEQ ID NO:90)

5

Equivalents

Those skilled in the art will recognize, or be able
 to ascertain, using no more than routine experimentation,
 10 many equivalents to the specific embodiments of the
 invention described herein. Such equivalents are intended
 to be encompassed by the following claims.